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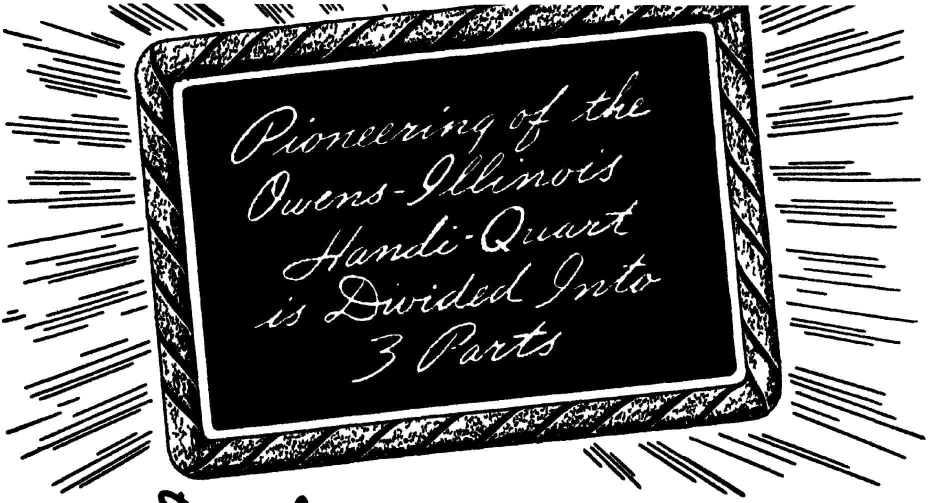

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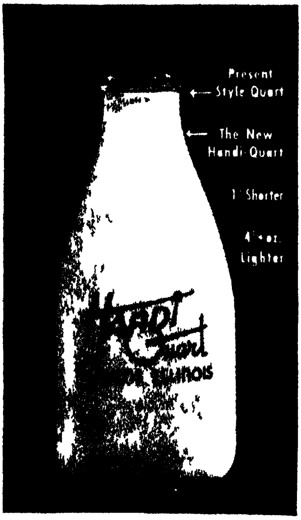

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OF
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VOLUME XXV

JANUARY, 1942, TO DECEMBER, 1942

1942
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ERRATA

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Page 635: *The fourth paragraph should read* The calf seems to be able to utilize rather diverse mixtures of plant products. In the popular bulletin of Herman (105) are recommended mixtures varying from 30 to 50 per cent in corn meal, from 25 to 30 in ground oats, from 20 to 30 per cent in wheat bran and from 10 to 25 per cent in linseed oil meal.

Page 648: *The second reference should read* (184) MORRISON, F. B., HULCE, R. S., AND HUMPHREY, G. C. Wis. Agr. Expt. Sta. Bul. 362, p. 96–99. 1924.

Page 648: *The first reference should read* (183) MORRISON, F. B., HULCE, R. S., AND HUMPHREY, G. C. Wis. Agr. Expt. Sta. Bul. 339, p. 134. 1922.

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THE EVALUATION OF FLAVOR DEFECTS OF BUTTER, CHEESE, MILK AND ICE CREAM AS DESIGNATED BY DAIRY PRODUCTS JUDGES

G. M. TROUT, CH., P. A. DOWNS, M. J. MACK,* E. L. FOUTS AND C. J. BABCOCK
Committee on Judging Dairy Products, A.D.S.A.

The American Dairy Science Association at its annual meeting in Burlington, Vermont, June, 1941, approved the recommendations of the Score Card Committee (1) relative to changes in the milk and ice cream score

TABLE 1

Range and average of judges' flavor scores of butter designated as having certain intensities of off-flavor

Flavor criticism	Range and average score* for butter flavor when the intensity of the defect was								
	Slight			Distinct			Strong		
	No.	Range	Av.	No	Range	Av.	No.	Range	Av.
Acidy	32	35.0–37.5	36.2	32	33.0–36.5	35.2	32	31.0–36.0	34.0
Bitter	31	33.0–37.0	35.5	31	32.0–36.0	34.5	29	31.5–35.0	33.4
Briny	31	34.5–38.0	36.5	31	34.0–37.0	35.6	30	32.3–36.5	33.5
Cheesy	32	32.0–35.0	34.0	32	31.0–34.0	32.9	29	31.0–33.0	31.9
Coarse	31	36.0–38.5	37.2	30	35.0–37.5	36.3	24	34.0–36.5	35.4
Cooked	32	36.0–38.0	37.5	32	35.0–38.0	36.6	28	32.8–37.0	35.6
Cowy	28	34.0–38.0	35.6	28	33.0–36.0	34.3	28	31.0–35.0	33.3
Feed	32	35.0–38.0	37.1	32	34.0–37.0	35.9	30	32.3–36.0	34.7
Fishy	32	32.0–37.0	33.2	31	31.0–33.5	31.9	20	31.0–32.0	31.4
Flat	31	37.0–38.0	37.4	28	36.0–38.0	36.6	23	34.5–37.0	36.0
Garlic	32	32.0–35.0	33.2	31	31.0–34.0	32.1	22	31.0–33.0	31.5
Gasoline	27	31.0–35.0	32.6	20	31.0–35.0	31.9	16	31.0–35.0	31.4
Malty	31	35.0–37.5	35.9	31	34.0–37.0	34.9	29	32.0–36.0	33.9
Metallic	31	33.0–36.0	34.6	31	31.0–36.0	33.5	28	31.0–34.0	32.5
Musty	30	33.0–36.0	34.9	30	32.5–35.0	33.8	30	31.0–33.0	32.6
Neutralizer	32	34.0–37.0	35.8	32	33.0–36.0	34.7	32	31.0–35.0	33.5
Oily	30	34.0–36.5	34.8	31	32.0–36.0	33.8	30	31.0–34.0	32.5
Old cream	32	35.0–37.0	36.0	32	33.8–36.0	34.8	32	32.0–35.0	33.5
Rancid	31	31.0–35.0	33.4	28	31.0–34.0	32.2	21	31.0–33.0	31.5
Storage	32	33.5–37.5	36.5	31	32.3–36.5	35.4	32	31.3–36.0	34.2
Tallowy	28	31.0–36.0	34.2	28	31.0–35.0	33.0	25	31.0–34.0	31.9
Unclean	31	33.0–37.0	35.1	31	32.0–36.0	33.8	29	31.0–35.0	32.6
Weedy	31	33.0–36.5	35.2	30	32.5–36.0	34.1	29	31.0–35.0	32.6
Woody	31	33.0–37.5	35.9	31	32.0–37.0	34.9	30	31.0–36.0	33.8
Yeasty	31	33.0–36.5	34.2	31	32.0–35.5	33.1	31	31.0–35.0	32.1

* Normal range of score on flavor 31 to 39.

Received for publication Feb. 16, 1942.

* Deceased Feb. 9, 1942.

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TABLE 2

Range and average of judges' flavor scores of cheese designated as having certain intensities of off-flavor

Flavor criticism	Range and average score* for cheese flavor when the intensity of the defect was								
	Slight			Distinct			Strong		
	No.	Range	Av.	No.	Range	Av.	No.	Range	Av.
Acidy	33	36.0–40.0	38.3	34	35.0–39.0	37.1	31	35.0–38.0	36.1
Bitter	34	35.0–40.0	37.8	31	35.0–39.0	36.7	26	35.0–38.0	35.8
Cowy	30	35.0–40.0	37.9	28	35.0–39.0	37.1	25	35.0–39.0	36.1
Feed	32	36.0–40.8	38.9	32	36.0–39.0	37.8	29	35.0–38.0	36.5
Fermented	32	35.5–39.5	37.5	31	35.0–38.0	36.4	23	35.0–37.0	35.5
Flat	34	38.0–41.0	39.5	33	37.0–40.0	38.7	30	35.0–39.0	37.8
Fruity	33	36.0–40.0	37.8	32	35.0–39.0	36.6	27	35.0–38.0	35.7
Heated	31	37.0–42.0	39.1	31	36.0–41.0	38.1	31	35.0–40.0	37.0
Moldy	31	35.0–40.0	37.2	30	35.0–37.0	36.1	24	35.0–38.0	35.4
Rancid	32	35.0–38.0	36.6	25	35.0–37.0	35.8	18	35.0–36.0	35.1
Unclean	33	35.0–39.0	37.7	30	35.0–38.0	36.7	25	35.0–37.0	35.5
Weedy	32	36.0–40.0	37.9	32	35.0–39.0	36.8	27	35.0–38.0	35.8
Yeasty	32	35.0–39.0	37.2	30	35.0–38.0	36.1	22	35.0–37.0	35.3

* Normal range of score on flavor 35 to 42.

TABLE 3

Range and average of judges' flavor scores of milk designated as having certain intensities of off-flavor

Flavor criticism	Range and average score* for milk flavor when the intensity of the defect was								
	Slight			Distinct			Strong		
	No.	Range	Av.	No.	Range	Av.	No.	Range	Av.
Bitter	37	29.5–38.0	34.3	37	25.0–36.0	30.9	32	25.0–34.0	27.8
Cooked	39	36.5–40.5	37.7	38	33.0–39.0	36.9	37	29.5–38.0	34.9
Cowy	38	30.0–39.0	36.0	38	25.0–36.5	32.8	35	25.0–34.0	28.2
Disinfectant	37	25.0–38.0	32.0	29	25.0–36.0	29.4	18	25.0–34.0	25.9
Feed	38	36.0–40.0	38.5	38	30.0–38.0	35.5	37	25.0–37.0	31.0
Flat	38	38.0–40.5	39.2	36	36.0–39.0	37.7	32	32.0–38.0	36.1
Garlic; onion	37	25.0–37.0	32.3	32	25.0–35.0	28.6	20	25.0–31.0	25.6
High acid	36	25.0–37.0	31.0	29	25.0–34.0	27.6	14	25.0–31.0	26.0
Malty	38	26.5–40.0	35.5	37	25.0–38.0	31.9	33	25.0–36.0	28.7
Metallic	36	30.0–38.0	35.5	36	25.0–35.0	31.4	31	25.0–33.0	27.7
Musty	35	30.0–38.0	34.5	35	25.0–38.0	30.8	31	25.0–31.0	26.9
Oxidized	38	33.0–39.0	35.6	38	25.0–37.0	31.7	34	25.0–34.0	27.3
Rancid	38	25.0–36.0	31.8	31	25.0–34.0	27.8	18	25.0–26.0	25.1
Salty	37	31.5–38.5	36.4	37	28.0–37.0	33.9	37	25.0–36.0	30.0
Unclean	37	25.0–38.0	34.7	36	25.0–36.0	30.7	30	25.0–33.0	26.9
Weedy	37	25.0–38.8	33.9	35	25.0–36.0	30.9	31	25.0–33.0	27.5

* Basis of 45. The committee on score cards A.D.S.A., C. J. Babcock, Ch., suggested the following guide in scoring flavor:

''Excellent: 40 and above; no criticism.
Good: 37 to 40; lacking special high flavor, flat, very slight feed, slight cooked.
Fair: 34 to 37; cooked, feed, salty, slight cowy, slight oxidized.
Poor: 25 to 34; strong feed, weedy, bitter, strong, musty, cowy, oxidized, very slight rancid.
Bad: 25 to below; strong cowy, high acid.
0; sour, putrid, or any flavor sufficiently strong to render the milk unfit for market purposes.''

Normal range of score on flavor 25 to 40.

TABLE 4

Range and average of judges' flavor scores of ice cream designated as having certain intensities of off-flavor

Flavor criticism	Range and average score* for ice cream flavor when the intensity of the defect was								
	Slight			Distinct			Strong		
	No.	Range	Av.	No.	Range	Av.	No.	Range	Av.
Cooked	34	38.0–40.0	39.2	34	36.0–39.0	37.6	33	32.0–39.0	35.8
Egg	32	37.0–39.7	38.9	34	34.0–39.0	37.6	32	31.0–38.0	35.9
Feed	30	34.0–39.5	37.4	30	33.5–38.5	35.6	29	31.0–37.5	33.2
High acid	34	33.0–38.0	35.9	33	31.0–36.5	33.5	29	31.0–36.0	32.0
Lacks fine flavor	33	38.0–40.0	39.3	31	36.0–40.0	38.3	29	34.5–39.0	37.3
Lacks flavoring	33	38.0–40.0	39.2	33	37.0–39.0	38.1	30	34.0–39.0	37.1
Lacks freshness	33	35.0–40.0	38.8	34	34.0–38.5	37.4	29	32.0–38.0	36.0
Lacks sweetness	33	38.0–40.0	39.1	34	36.0–39.5	38.1	30	33.0–39.0	36.9
Metallic	32	33.0–39.0	36.3	32	31.0–36.5	34.4	29	31.0–36.8	32.7
Neutralizer	35	31.0–39.0	35.1	33	31.0–37.0	33.1	31	31.0–34.0	31.5
Old ingredient	34	32.5–38.0	36.4	34	31.5–36.0	34.2	31	31.0–35.5	32.0
Oxidized	34	34.0–38.0	36.3	34	32.0–36.0	34.2	33	31.0–34.0	31.8
Rancid	32	31.0–37.0	34.1	31	31.0–34.0	32.4	24	31.0–33.0	31.2
Salty	33	31.0–39.5	37.0	32	32.0–38.0	35.0	31	31.0–36.0	32.6
Storage	34	34.5–40.0	37.0	34	32.5–38.5	35.0	34	31.0–36.0	32.9
Unclean	33	31.0–38.0	34.9	32	31.0–36.0	33.0	28	31.0–33.5	31.5
Unnatural flavoring	34	36.5–39.0	37.2	34	32.0–37.5	35.2	33	31.0–37.0	33.4

* Basis of 45. The following guide having the same grouping as used previously in the national contest but evaluated on the basis of 45 instead of 50 was suggested for use of the judges:

	Flavor	
‘‘Score 39.5–37.5	Cooked Egg Lacks fine flavor Lacks flavoring	Lacks freshness Lacks sweetness Too high flavor Too sweet
Score 37.5–34.5	Cooked Feed High acid Metallic Old ingredient	Oxidized Salty Storage Unnatural flavoring
Score 34.5–31.0	Feed High acid Neutralizer Old ingredient Oxidized	Rancid Salty Storage Unclean’’

Normal range of score on flavor 31 to 40.

cards. Among other changes, the points allowed for flavor were increased from 25 to 45 on the milk score card and decreased from 50 to 45 on the ice cream score card. Thus the score cards each for butter, cheese, milk and ice cream now carry the same weights for flavor, 45 points.

Flavor commands such a high relative importance on each of the score cards that, inasmuch as revaluation of flavor defects was now necessary in scoring milk and ice cream due to changes in those score cards, it seemed desirable for the sake of uniformity of judging throughout the country to evaluate flavor defects for all the four major dairy products. Consequently, some 45 or 50 coaches of dairy products judging teams and dairy products judges, all trained men in dairy products judging were asked to evaluate definite intensities of flavors appearing on the contestants' dairy products judging contest score cards. The response was most gratifying, not only from the number of evaluations received but from the fact that the evaluations represented practically 100 per cent independent judgment. Those giving flavor evaluations for one or more of the four major dairy products

TABLE 5

Average score of certain flavors occurring in three or more products

Flavor	Average flavor score* in											
	Butter			Cheese			Milk			Ice cream		
	Slight	Distinct	Strong	Slight	Distinct	Strong	Slight	Distinct	Strong	Slight	Distinct	Strong
Non-selected judges												
Bitter	35.5	34.4	33.4	37.8	36.7	35.8	34.3	30.9	27.8			
Cooked	37.5	36.6	35.6				37.7	36.9	34.9	39.2	37.6	35.8
Cowy	35.5	34.3	32.3	37.9	37.1	36.1	36.0	32.8	28.2			
Feed	37.1	35.9	34.7	38.9	37.8	36.5	38.5	35.5	31.0	37.4	35.6	33.2
Flat	37.3	36.6	36.0	39.5	38.7	37.8	39.2	37.7	36.1			
Metallic	34.6	33.5	32.5				35.5	31.4	27.7	36.3	34.4	32.7
Rancid	33.4	32.2	31.5	36.6	35.8	35.1	31.8	27.8	25.1	34.1	32.4	31.2
Unclean	35.1	33.8	32.6	37.7	36.7	35.5	34.7	30.7	26.9	34.9	33.0	31.5
Selected judges												
Bitter	35.8	34.4	31.0	38.5	37.2	35.6	35.6	30.7	25.2			
Cooked	37.9	37.0	35.7				39.1	37.1	34.4	39.1	37.5	35.1
Cowy	35.8	34.7	33.3	37.8	36.7	35.3	36.1	31.1	25.4			
Feed	37.6	36.2	35.0	38.7	37.6	36.5	38.8	35.7	31.0	37.5	35.7	33.6
Flat	37.8	36.9	36.3	39.5	38.9	38.4	39.5	38.4	36.6			
Metallic	34.4	33.2	31.5				36.4	30.3	24.0	36.6	34.6	32.6
Rancid	33.0	31.7	32.2	36.8	35.7	34.8	33.1	27.1	19.2	35.3	33.2	31.0
Unclean	35.8	34.3	33.0	38.1	36.9	33.2	33.1	29.1	22.6	35.3	33.6	31.2

* Basis of 45 points for flavor per product.

are as follows: E. O. Anderson, C. J. Babcock, H. A. Bendixen, F. W. Bennett, H. C. Boxell, W. C. Brown, L. H. Burgwald, W. B. Combs, S. T. Coulter, A. C. Dahlberg, F. J. Doan, L. R. Dowd, P. A. Downs, L. S. Edwards, C. W. England, J. H. Erb, N. E. Fabricius, E. L. Fouts, I. A. Gould, E. S. Guthrie, T. B. Harrison, J. L. Henderson, H. B. Henderson, E. O. Herreid, F. H. Herzer, C. Jensen, D. V. Josephson, W. A. Krienke, H. L. Lindquist, M. J. Mack, W. H. Martin, A. V. Moore, H. C. Moore, J. A. Nelson, J. A. Newlander, H. C. Olsen, M. G. Pederson, W. V. Price, K. M. Renner, H. A. Smallfield, L. C. Thomsen, C. C. Totman, P. H. Tracy, S. L. Tuckey, W. F. Widdefield, H. L. Wilson, and G. H. Wilster.

Percentage distribution studies were made of the scores allotted by these judges for the intensities of flavors of butter, cheese, milk and ice cream. When a judge gave a range of score rather than a specific value for a certain intensity of flavor the average value was tabulated. When the score was given in 0.5 of a point the number was recorded as the next lower whole number. Generally, a specific whole number was given for each flavor defect. In case the flavor score given was below the normal range designated, or was indicated as "no score," the evaluation was disregarded in the tabulation.

TABLE 6

Judges of butter, cheese, milk and ice cream selected by 39 coaches for special tabulation of their evaluations on various intensities of flavor defects

Product	Name of judge	College, Department or University represented	Ballots cast for each judge	
			No.	Per cent
Butter	Coulter, S. T.	Minnesota	28	71.8
	Edwards, L. S.	U. S. Dept. Agr.	26	66.7
	Fabricius, N. E.	Iowa	31	79.5
	Guthrie, E. S.	Cornell	21	53.8
	Herzer, F. H.	Mississippi	16	41.0
Cheese	Anderson, E. O.	Connecticut	13	33.3
	Coulter, S. T.	Minnesota	12	30.8
	Harrison, T. B.	Tennessee	12	30.8
	Herzer, F. H.	Mississippi	13	33.3
	Thomsen, L. C.	Wisconsin	26	66.7
	Wilson, H. L.	U. S. Dept. Agr.	34	87.2
Milk	Anderson, E. O.	Connecticut	14	35.9
	Babcock, C. J.	U. S. Dept. Agr.	33	84.6
	Burgwald, L. H.	Ohio	26	66.7
	Doan, F. J.	Pennsylvania	21	53.8
	Gould, I. A.	Michigan	24	61.5
Ice Cream	Dahlberg, A. C.	N. Y. (Geneva) Agr. Expt. Sta.	22	56.4
	Erb, J. H.	Ohio	24	61.5
	Mack, M. J.	Massachusetts	20	51.3
	Martin, W. H.	Kansas	23	59.0
	Tracy, P. H.	Illinois	28	71.8

These distributions of evaluations for various intensities of flavor defects for each product are too extensive for inclusion in this paper. Consequently, they were incorporated in a separate report (2) which was distributed to the various judges and is yet available to those interested. The data show that in some cases, wide differences occur in the judges' evaluations of some flavors and in other cases there is a comparatively close agreement as to what should constitute the correct flavor score. These data indicated also the percentage of the judges who favored specific evaluations. Generally the higher percentage of the judges' evaluations were centered near the average evaluation.

THE AVERAGE SCORE FOR EACH FLAVOR DEFECT IN BUTTER, CHEESE, MILK, AND ICE CREAM

The average score for each flavor defect of butter, cheese, milk, and ice cream, the range in scores, and the number of judges involved are included in tables 1, 2, 3, and 4 respectively. From these tables, which are worthy of very close study, data relative to flavors appearing on the score cards for each of the four products were taken and assembled in table 5. A study of these data show that a tendency existed to cut consistently specific flavor defects on one product more than on others. Especially was this true in some of the more serious flavor defects on the milk score card. The character of the product doubtless has an important bearing on the score given a certain intensity of flavor. For example, the volume of flavor in cheese as contrasted to milk may cause a slight intensity of flavor in cheese seem to be a strong intensity of off-flavor in milk.

TABLE 7

Range and average flavor score of butter designated as having certain intensities of off-flavor as given by selected judges

Flavor criticism	Range and average score for butter when the intensity of the defect was					
	Slight		Distinct		Strong	
	Range	Av.	Range	Av.	Range	Av.
Acidy	36.0–37.0	36.4	34.0–36.5	35.1	33.0–36.0	34.0
Bitter	35.0–36.0	35.8	34.0–35.0	34.4	32.0–34.0	33.0
Briny	36.5–38.0	37.1*	35.0–37.0	36.2*	33.0–36.5	35.1*
Cheesy	33.0–34.0	33.8	32.0–33.0	32.6	30.0–32.0	31.4
Coarse	37.0–38.0	37.2	35.0–37.0	36.0*	34.0–36.5	35.1*
Cooked	37.5–38.0	37.9	37.0	37.0	35.0–36.0	35.7†
Cowy	35.0–37.0	35.7†	34.0–36.0	34.7†	32.0–35.0	33.3
Feed	37.0–38.0	37.6	35.0–37.0	36.2	33.0–36.0	35.0
Fishy	32.0–34.0	32.6	30.0–33.0	31.2	30.0–32.0	31.0†
Flat	37.5–38.0	37.8*	36.0–37.5	36.9*	35.0–37.0	36.3†
Garlic	32.0–33.0	32.6	30.0–32.0	31.2	30.0–31.0	30.5*
Gasoline	30.0–34.0	32.0*	30.0–33.0	31.3†	31.0–32.0	31.5‡
Malty	36.0–37.0	36.2	34.0–36.0	35.0	32.0–35.5	33.8
Metallic	34.0–35.0	34.4	33.0–34.0	33.2	30.0–33.0	31.5*
Musty	34.0–36.0	35.2	33.0–35.0	34.0	31.0–33.0	32.4
Neutralizer	35.0–36.5	35.9	33.0–36.0	34.5	32.0–35.0	33.3
Oily	34.0–36.0	34.6	32.0–35.0	33.3	31.0–34.0	32.2
Old cream	35.0–37.0	35.8	33.0–35.0	34.1	32.0–35.0	33.6
Rancid	32.0–35.0	33.0†	30.0–34.0	31.7†	31.0–33.0	32.2†
Storage	37.0–37.5	37.1	35.0–36.5	35.8*	33.0–36.0	35.0
Tallowy	32.0–36.0	34.0†	31.0–35.0	32.7†	31.0–34.0	32.7
Unclean	35.0–36.0	35.8	34.0–35.5	34.3	32.0–35.0	33.0
Weedy	35.0–36.0	35.6	33.0–35.0	34.1	30.0–34.0	32.1*
Woody	36.0–37.0	36.2	35.0–35.5	35.1	34.0–35.0	34.2
Yeasty	33.0–35.0	34.0	32.0–34.0	33.0	32.0–33.0	32.3*

* Average of four scores, one not given.
† Average of three scores, two not given.
‡ Average of two scores, three not given.

TABLE 8

Range and average flavor score of cheese designated as having certain intensities of off-flavor as given by selected judges

Flavor criticism	Range and average score for cheese flavor when the intensity of the defect was					
	Slight		Distinct		Strong	
	Range	Av.	Range	Av.	Range	Av.
Acidy	38.0–39.5	38.5	37.0–38.5	37.3	35.0–36.5	35.8
Bitter	38.0–39.5	38.5	36.0–38.5	37.2	35.0–36.5	35.6
Cowy	36.0–38.5	37.8	35.0–37.5	36.7	35.0–36.0	35.3
Feed	38.0–39.5	38.7	37.0–38.5	37.6	36.0–37.0	36.5
Fermented	36.0–38.0	37.3	35.0–37.0	36.2	34.0–35.5	35.0
Flat	39.0–41.0	39.5	38.0–40.0	38.9	37.0–39.0	38.4
Fruity	37.0–39.0	37.8	36.0–38.0	36.7	35.0–37.0	35.5
Heated	38.5–39.0	38.9	37.0–38.0	37.8	36.0–38.0	37.0
Moldy	37.0–38.0	37.2	35.0–36.5	35.9	35.0–35.5	35.1
Rancid	36.0–37.5	36.8	35.0–36.3	35.7	34.0–35.0	34.8
Unclean	37.5–39.0	38.1	36.5–37.0	36.9	35.0–35.5	35.1
Weedy	37.0–39.0	37.9	36.0–37.5	36.8	34.0–36.0	35.3
Yeasty	36.0–38.0	37.1	35.0–37.0	36.0	34.0–36.0	35.0

SELECTION OF PANEL OF JUDGES FOR EVALUATING FLAVOR DEFECTS OF BUTTER, CHEESE, MILK AND ICE CREAM

From the wide range noted in flavor scores in butter, cheese, milk and ice cream as given by the coaches and judges as a whole, it seemed desirable to ascertain what values selected judges might place upon these same flavors.

TABLE 9

Range and average flavor score of milk having certain intensities of off-flavor as given by selected judges

Flavor criticism	Range and average score for milk flavor when the intensity of the defect was					
	Slight		Distinct		Strong	
	Range	Av.	Range	Av.	Range	Av.
Bitter	34.0–37.5	35.6	25.0–34.0	30.7	12.0–30.0	25.2
Cooked	38.0–40.0	39.1	36.0–38.5	37.1	29.5–37.0	34.4
Cowy	35.5–37.0	36.1	25.0–34.0	31.1	12.0–30.0	25.4
Disinfectant	33.0–35.0	34.0	25.0–30.0	28.1	0.0–25.0	19.2
Feed	38.0–40.0	38.8	35.0–36.0	35.7	29.0–33.0	31.0
Flat	39.0–40.0	39.5	38.0–39.0	38.4	34.0–38.0	36.6*
Garlic; onion	25.0–35.5	32.5	12.0–30.0	25.7	0.0–26.0	19.2
High acid	30.0–37.0	33.3	25.0–30.0	26.9	12.0–26.0	21.6
Malty	36.0–37.0	36.2	25.0–33.0	29.7	12.0–30.0	24.0
Metallic	36.0–37.0	36.4	25.0–34.0	30.3	12.0–32.0	24.0
Musty	34.0–36.0	35.1	25.0–32.0	29.5	0.0–25.0	20.2
Oxidized	34.0–36.5	35.5	25.0–32.0	28.9	12.0–26.0	21.6
Rancid	30.0–35.5	33.1	25.0–30.0	27.1	0.0–26.0	19.2
Salty	35.0–38.5	37.4	30.0–37.0	34.3	25.0–36.0	30.1
Unclean	34.0–36.0	35.1	25.0–34.0	29.1	12.0–30.0	22.6
Weedy	34.0–37.0	35.5	25.0–34.0	29.9	12.0–32.0	24.0

* Average of four scores, one not given.

Consequently, the coaches were invited to name their choices of five judges each of butter, cheese, milk and ice cream whose flavor evaluations would be tabulated separately. A total of 39 coaches responded with their nominations. The five coaches in each product securing the most ballots were selected as the board of judges whose flavor scores were classified as a unit. The names of the judges selected are presented in table 6. In butter, milk, and ice cream, little competition aside from those selected was manifest for a place on the board. However the nominations for cheese judges were quite scattered. In fact, it was necessary to select a battery of six rather than five to constitute the board. Inasmuch as the majority of the judges selected had already placed a valuation on the flavor defects of the various products, a second evaluation was not solicited, but those previously given were reclassified.

TABLE 10

Range and average flavor score of ice cream designated as having certain intensities of off-flavor as given by selected judges

Flavor criticism	Range and average score for ice cream flavor when the intensity of the defect was					
	Slight		Distinct		Strong	
	Range	Av.	Range	Av.	Range	Av.
Cooked	39.0–39.5	39.1	37.0–38.5	37.5	32.0–37.5	35.1
Egg	38.0–39.5	38.9	37.0–39.0	37.8	35.0–38.0	36.6
Feed	37.0–39.0	37.5	35.0–37.0	35.7	32.0–35.0	33.6
High acid	35.0–37.5	36.1	33.0–35.0	33.8	31.0–37.0	32.4
Lacks fine flavor	39.0–40.0	39.4	38.0–39.0	38.7	37.5–39.0	38.1*
Lacks flavoring	39.0–40.0	39.4	38.0–39.0	38.4	37.0–38.0	37.5*
Lacks freshness	39.0–39.5	39.2	38.0–38.5	38.2	37.0–37.5	37.3*
Lacks sweetness	39.0–39.5	39.2	36.0–39.0	37.8	33.0–38.0	36.0*
Metallic	35.0–37.0	36.6	33.0–36.0	34.6	31.0–35.0	32.6
Neutralizer	34.0–38.0	35.4	32.0–36.0	33.6	31.0–34.0	31.8
Old ingredient	36.0–38.0	37.1	34.0–36.0	35.2	31.0–34.5	32.7
Oxidized	36.0–37.0	36.8	34.0–35.0	34.6	31.0–33.0	31.8
Rancid	34.0–37.0	35.3	32.0–34.0	33.2	31.0	31.0
Salty	37.0–39.0	37.7	34.0–37.0	35.4	31.0–35.0	32.4
Storage	37.0–40.0	37.9	34.0–38.0	35.8	31.0–36.0	33.3
Unclean	34.0–37.0	35.3	33.0–35.0	33.6	31.0–32.0	31.2
Unnatural flavoring	36.0–39.0	37.3	35.0–37.0	35.8	33.0–35.0	34.3

* Average of four scores, one not given.

The range and average scores placed on the flavors of the several products by the selected judges are given in tables 7, 8, 9, and 10. A study of these tables in comparison with tables 1, 2, 3, and 4 shows a marked closeness in score per flavor per product between the two averages. Probably more conservatism in making cuts for slight flavor defects was manifest in the group of selected judges than was evident among the coaches as a whole. On the other hand, they appeared to be more drastic in making cuts when the flavor defect was such as to question seriously the marketability of the product.

CLASSIFICATION OF FLAVORS ACCORDING TO SCORE

Since it was shown (3) that the judge who could criticize the samples fairly accurately might be able to score reliably as well, but not *vice versa,* it seemed desirable to classify the off-flavors of butter, cheese, milk, and ice

TABLE 11

Classification of off-flavors of butter according to its market grade

Approximate total score*	Flavor criticism	Intensity of flavor defect	Average score given by selected judges
93			38.0† and above
92	Cooked	Slight	37.9
	Flat	"	37.8
	Feed	"	37.6
	Coarse	"	37.2
	Briny	"	37.1
	Storage	"	37.1
	Cooked	Distinct	37.0
91	Flat	"	36.9
	Acidy	Slight	36.4
	Flat	Strong	36.3
	Feed	Distinct	36.2
	Malty	Slight	36.2
	Woody	"	36.2
	Briny	Distinct	36.2
	Coarse	"	36.0
90	Neutralizer	Slight	35.9
	Bitter	"	35.8
	Old cream	"	35.8
	Unclean	"	35.8
	Storage	Distinct	35.8
	Cooked	Strong	35.7
	Cowy	Slight	35.7
	Weedy	"	35.6
	Musty	"	35.2
	Briny	Strong	35.1
	Coarse	"	35.1
	Acidy	Distinct	35.1
	Woody	"	35.1
	Feed	Strong	35.0
	Malty	Distinct	35.0
	Storage	Strong	35.0
89	Cowy	Distinct	34.7
	Oily	Slight	34.6
	Neutralizer	Distinct	34.5
	Bitter	"	34.4
	Metallic	Slight	34.4
	Unclean	Distinct	34.3
93		. .	38.0† and above
89	Woody	Strong	34.2
	Old cream	Distinct	34.1
	Weedy	"	34.1
	Acidy	Strong	34.0
	Musty	Distinct	34.0
	Tallowy	Slight	34.0
	Yeasty	"	34.0
88	Cheesy	Slight	33.8
	Malty	Strong	33.8
	Old cream	"	33.6
	Cowy	"	33.3
	Neutralizer	"	33.3
	Oily	Distinct	33.3
	Metallic	"	33.2
	Bitter	Strong	33.0
	Rancid	Slight	33.0
	Unclean	Strong	33.0
	Yeasty	Distinct	33.0
87	Tallowy	Distinct	32.7
	Tallowy	Strong	32.7
	Cheesy	Distinct	32.6
	Fishy	Slight	32.6
	Garlic	"	32.6
	Musty	Strong	32.4
	Yeasty	"	32.3
	Oily	"	32.2
	Rancid	"	32.2
	Weedy	"	32.1
	Gasoline	Slight	32.0
86	Rancid	Distinct	31.7
	Gasoline	Strong	31.5
	Metallic	"	31.5
	Cheesy	"	31.4
	Gasoline	Distinct	31.3
	Fishy	"	31.2
	Garlic	"	31.2
	Fishy	Strong	31.0
	Garlic	"	30.5

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* Other items on the score card rating a perfect score.

† From Rules of Students' National Contest in the Judging of Dairy Products, 1942.

cream into groups according to the seriousness of the defect as shown by the average score given by the selected judges, in order to enable the prospective judge to correlate more easily the flavor, its score and its market class. Consequently the off-flavors of butter, cheese, milk and ice cream were accordingly grouped as shown in tables 11, 12, 13 and 14.

By this classification the prospective judge may recognize at a glance the group to which flavor defects belong as well as their approximate scores. Such a grouping also gives a rating of the seriousness of the various flavors encountered.

TABLE 12

Classification of off-flavors of cheese according to a suggested grouping

Suggested flavor classes and score	Flavor criticisms	Intensity of flavor defect	Average score given by selected judges
Excellent (40–45)	...	...	40.0* and above
Good (38.5–39.5)	Flat	Slight	39.5
	Flat	Distinct	38.9
	Heated	Slight	38.9
	Feed	“	38.7
	Acidy	“	38.5
	Bitter	“	38.5
Fair (37–38.5)	Flat	Strong	38.4
	Unclean	Slight	38.1
	Weedy	“	37.9
	Cowy	“	37.8
	Heated	Distinct	37.8
	Fruity	Slight	37.8
	Feed	Distinct	37.6
	Acidy	“	37.3
	Fermented	Slight	37.3
	Bitter	Distinct	37.2
	Moldy	Slight	37.2
	Yeasty	“	37.1
	Heated	Strong	37.0
Poor (36–37)	Unclean	Distinct	36.9
	Rancid	Slight	36.8
	Weedy	Distinct	36.8
	Cowy	“	36.7
	Fruity	“	36.7
	Feed	Strong	36.5
	Fermented	Distinct	36.2
	Yeasty	“	36.0
Bad (35–36)	Moldy	“	35.9
	Acidy	Strong	35.8
	Rancid	Distinct	35.7
	Bitter	Strong	35.6
	Fruity	“	35.5
	Cowy	“	35.3
	Weedy	“	35.3
	Unclean	“	35.2
	Moldy	“	35.1
	Fermented	“	35.0
	Yeasty	“	35.0
	Rancid	“	34.8

* From Rules for Students' National Contest in the Judging of Dairy Products, 1942.

TABLE 13

Classification of flavors of milk according to the class of milk

Class of milk* with approximate score	Flavor criticism	Intensity of flavor	Average score given by selected judges
Excellent (40–45)			40* and above
Good (37–40)	Flat	Slight	39.5
	Cooked	"	39.1
	Feed	"	38.8
	Flat	Distinct	38.4
	Salty	Slight	37.4
	Cooked	Distinct	37.1
Fair (34–37)	Flat	Strong	36.6
	Metallic	Slight	36.4
	Malty	"	36.2
	Cowy	"	36.1
	Feed	Distinct	35.7
	Bitter	Slight	35.6
	Oxidized	"	35.5
	Weedy	"	35.5
	Musty	"	35.1
	Unclean	"	35.1
	Cooked	Strong	34.4
	Salty	Distinct	34.3
	Disinfectant	Slight	34.0
Poor (25–34)	High acid	Slight	33.3
	Rancid	"	33.1
	Garlic; onion	"	32.5
	Cowy	Distinct	31.1
	Feed	Strong	31.0
Excellent (40–45)			40* and above
Poor Cont'd. (25–34)	Bitter	Distinct	30.7
	Metallic	"	30.3
	Salty	Strong	30.1
	Weedy	Distinct	29.9
	Malty	"	29.7
	Musty	"	29.5
	Unclean	"	29.1
	Oxidized	"	28.9
	Disinfectant	"	28.1
	Rancid	"	27.1
	High acid	"	26.9
	Garlic; onion	"	25.7
	Cowy	Strong	25.4
	Bitter	"	25.2
Bad (25 or below)	Malty	Strong	24.0
	Metallic	"	24.0
	Weedy	"	24.0
	Unclean	"	22.6
	High acid	"	21.6
	Oxidized	"	21.6
	Musty	"	20.2
	Disinfectant	"	19.2
	Garlic; onion	"	19.2
	Rancid	"	19.2

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* Suggested by committee on score cards, A.D.S.A., C. J. Babcock, Ch.

TABLE 14

Classification of flavors of ice cream according to a suggested grouping

Suggested grouping for off-flavors	Flavor criticism	Intensity of flavor defect	Average score given by selected judges
Excellent (40–45)	.	. . .	40* and above
Good (39.5–37.5)	Lacks fine flavor	Slight	39.4
	Lacks flavoring	“	39.4
	Lacks freshness	“	39.2
	Lacks sweetness	“	39.2
	Cooked	“	39.1
	Egg	“	38.9
	Lacks fine flavor	Distinct	38.7
	Lacks flavoring	“	38.4
	Lacks freshness	“	38.2
	Lacks fine flavor	Strong	38.1
	Storage	Slight	37.9
	Egg	Distinct	37.8
	Lacks sweetness	“	37.8
	Salty	Slight	37.7
Fair (37.5–34.5)	Cooked	Distinct	37.5
	Feed	Slight	37.5
	Lacks flavoring	Strong	37.5
	Unnatural flavoring	Slight	37.3
	Lacks freshness	Strong	37.3
	Old ingredient	Slight	37.1
	Oxidized	“	36.8
	Egg	Strong	36.6
	Metallic	Slight	36.6
	High acid	“	36.1
	Lacks sweetness	Strong	36.0
	Storage	Distinct	35.8
	Unnatural flavoring	“	35.8
	Feed	“	35.7
Poor (34.5–31.0)	Neutralizer	Slight	35.4
	Salty	Distinct	35.4
	Rancid	Slight	35.3
	Unclean	“	35.3
	Old ingredient	Distinct	35.2
	Cooked	Strong	35.1
	Metallic	Distinct	34.6
	Oxidized	Distinct	34.6
	Unnatural flavoring	Strong	34.3
	High acid	Distinct	33.8
	Feed	Strong	33.6
	Neutralizer	Distinct	33.6
	Unclean	“	33.6
	Storage	Strong	33.3
	Rancid	Distinct	33.2
	Old ingredient	Strong	32.7
	Metallic	“	32.6
	High acid	“	32.4
	Salty	“	32.4
	Neutralizer	“	31.8
	Oxidized	“	31.8
	Unclean	“	31.2
	Rancid	“	31.0

* From Rules for Students' National Contest in the Judging of Dairy Products, 1942.

In studying the average score values it should be kept in mind that the smallest cut for flavor in the Students' National Contest in the Judging of Dairy Products is 0.5 of a point whereas the average value expressed here is in one-tenth of a point. These average values might be used only as a guide in setting up standards for teaching based upon one-half point cuts on the score card. It would seem highly advisable for the coach or judge of dairy products in working up a list of scores for his own personal use to follow at least some of the averages of the scores given by the selected judges, but of equal or more importance to determine the trend of thought of the selected judges and work up the chart of scores accordingly.

SUMMARY AND CONCLUSIONS

A study was made of the flavor scores of butter, cheese, milk, and ice cream as given by 47 trained judges and by a panel of five selected judges for each product.

The judgments of the selected judges were found for the most part to be within a narrower range than those of the group of judges as a whole.

A knowledge of the classes or groups of off-flavors of butter, cheese, milk, and ice cream as arranged from the numerical scores given by the selected judges would seem to be of material value, 1, to those interested in becoming proficient in dairy products judging; 2, in unifying and standardizing dairy products judging throughout the United States; and 3, in furnishing a common basis for recording and evaluating research in which flavor data are involved.

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CAROTENE IN CALF NUTRITION[1]

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The indispensable nature of vitamin A in the ration of dairy calves was shown by Jones, Eckles and Palmer (8) as early as 1926. Bechdel, Eckles and Palmer (1) reported similar findings the same year. Hart and Guilbert (7) found that a vitamin A deficiency developed in cattle under range conditions during unusually long seasons of dry feeding. Guilbert and Hart (4) reported that 26–33 micrograms of carotene from alfalfa per day per kilogram of body weight was sufficient to prevent or cure vitamin A deficiency in cattle. Guilbert, Miller and Hughes (5) also reported the minimum carotene requirement for cattle, sheep and swine to be between 25 and 30 micrograms per day per kilogram of body weight. The vitamin A requirement was found to be 6–8 micrograms daily per kilogram of body weight. However, data obtained by Halverson, Hostetler, Foster and Sherwood (6) indicated that the minimum daily carotene requirement of cattle was 43 to 55 I. U. per kilogram of body weight.

Moore (10) found that a carotene intake of about 16 micrograms per pound of body weight per day was sufficient to maintain plasma carotene at 0.2 microgram per milliliter, to prevent nyctalopia and to maintain a fair state of general health. When plasma carotene values fell below 0.13 microgram per milliliter, nyctalopia and papillary edema were found to follow. Converse and Meigs (2) concluded that because of the greater susceptibility of calves to various calf ailments during the first 3–4 months of life than at older ages they need a more generous allowance of vitamin A than at older ages. These workers concluded that the California standard (4) is less than one-third of the optimum for calves between 3 and 180 days of age. Ward, Bechdel and Guerrant (12, 13) found that 11 micrograms of carotene from a carotene concentrate per pound of body weight per day prevented vitamin A deficiency in Holstein calves. This requirement was also met by 14

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micrograms of carotene from alfalfa hay or by 33 micrograms of carotene from alfalfa molasses silage. It was also observed that the carotene requirements of some calves appeared to be slightly higher in cold than in mild or moderate weather. The present experiments are a continuation of the previous studies, using certain improvements in experimental technic.

EXPERIMENTAL

Thirty-four calves representing the Guernsey and Holstein breeds were used in these experiments to determine the minimum amount of carotene that would prevent vitamin A deficiency symptoms in dairy calves from the time of birth to 6–8 months of age. The experimental results obtained with 24 of these experimental subjects are reported at this time. All calves were removed from their dams at 2 to 4 days of age and were placed on reconstituted skimmilk at 4 to 7 days of age, except the first 6 calves which were kept on whole milk until 4 weeks of age. A ration composed of wheat bran, rolled oats, ground barley, linseed oil meal, soybean oil meal, molasses, steamed bone meal, salt and irradiated yeast was fed according to appetite. The above ration contained 13.0 per cent digestible crude protein and 72.2 per cent total digestible nutrients. Dried beet pulp, as the sole roughage, was fed according to appetite.

Calves were maintained in individual stalls in a well ventilated and well lighted calf barn. Although artificial heat was used in the barn at times, the temperature therein was not maintained as constant as was hoped, particularly during cold and windy weather. Sawdust was used as the bedding.

Alfalfa leaf meal of high quality was used as the principal source of carotene. It was weighed into gelatin capsules and fed with a balling gun. While the plan of the experiment was to keep each calf on a definite daily carotene intake (per pound of body weight), small variations in daily dosages did occur due to changes in the carotene content of the alfalfa leaf meal and to slight variations in body weight. Carotene assays on the alfalfa leaf meal were made by both the biological and the colorimetric methods.

The experimental animals were weighed at weekly intervals and the carotene intake adjusted according to changes in weight. The eyes of the calves were examined weekly by means of an opthalmoscope according to the method of Moore (9). A complete record was made of all other visible conditions that appeared to have any relation to a deficiency of carotene in the diet.

Blood carotene determinations were made every two weeks by a method developed for this work. This consisted in precipitating whole blood with methanol, extracting with petroleum ether, saponifying and then determining the carotene concentration by means of a photoelectric colorimeter. During the last 10 months of the experiment an aliquot from each petroleum ether extract was evaporated to dryness and the residue was taken up in isopropyl alcohol. Vitamin A readings were made at 328 millimicrons by means of an electronic photometer. While these data, in all probability, are only relative, they are of value in comparative studies.

The calves were slaughtered when removed from the experiment. A careful examination was made of the various organs for gross pathological conditions. Microscopic studies were conducted on tissues from the various organs. A summary of these observations has been published elsewhere (11).

DATA

Table 1 gives a summary of the experimental set-up employed and the results obtained with the different experimental animals. The season of the year during which each calf was on experiment should be considered in studying this table.

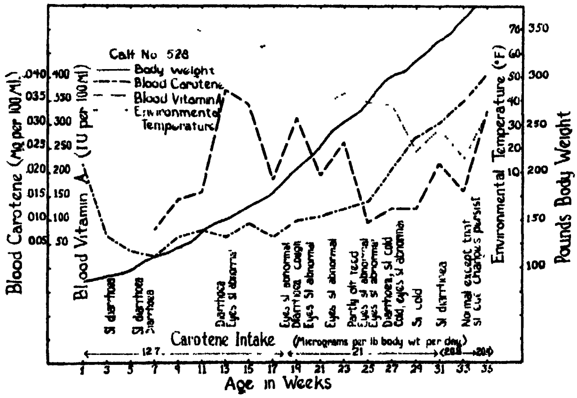

FIG. 1. Illustrating the relationship that was found to exist between environmental temperature, body weight changes, blood carotene, blood vitamin A level, and the general condition of calf No. 528.

Figures 1 and 2 illustrate with individual calves the relationship that was found to exist between environmental temperature, the body weight changes, the blood carotene level, the blood vitamin A level and the condition of the calf. Figure 3 illustrates further the relationship found between the carotene and the vitamin A levels in the blood and the environmental temperature, the carotene and vitamin A values being the averages from six animals.

DISCUSSION OF RESULTS

Examination of the eye by means of an opthalmoscope appeared to be the most sensitive method of detecting the effects of vitamin A deficiency. The chief defect of this method was due to changes produced in the eye by vitamin A deficiency which frequently persisted for weeks or even months after the carotene intake had been increased to an apparently adequate level.

The blood carotene and vitamin A levels seemed to indicate rather reliably the adequacy of the carotene intake, although here again individual variations were observed. The vitamin A level appeared to be a better indicator of an adequate intake of vitamin A than did the blood carotene level. This is what one might expect since it is vitamin A *per se* that is used by the body and not carotene. However, it appeared that when blood carotene fell below .0175 milligram per 100 milliliters of whole blood in Holstein calves, vitamin A deficiency symptoms were frequently found. Above this figure vitamin A deficiency symptoms were rarely observed. Such a value for Guernsey calves appeared to be somewhat higher. It seems undesirable at this time to attempt to set a minimum value for blood vitamin A because the values determined in these experiments may be only relative. However, Davis and Madsen (3) have set 25 micrograms of carotene and 16 micrograms of vitamin A per 100 milliliters of plasma as the critical level.

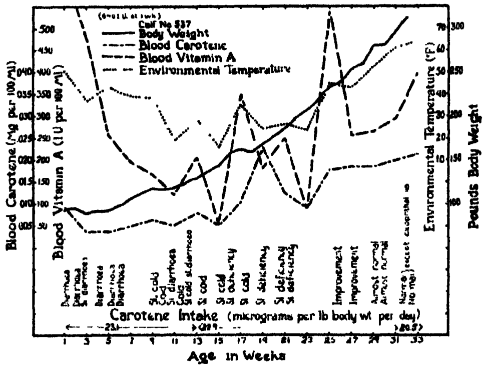

FIG. 2. Illustrating the relationship that was found to exist between environmental temperature, body weight changes, blood carotene, blood vitamin A level, and the general condition of calf No. 537.

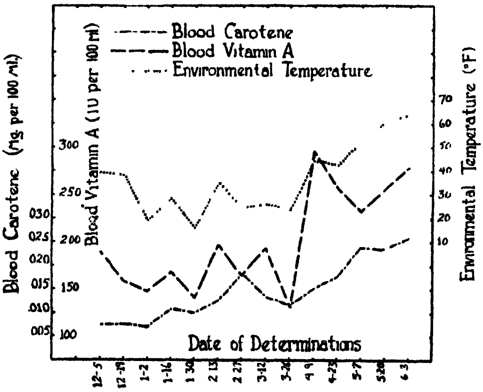

FIG. 3. Illustrates the relationship found to exist between environmental temperature, blood carotene and blood vitamin A (average values of six representative calves).

The observed effects of vitamin A deficiency in calves were similar to those previously reported by other workers. These included dilated pupils, bleached tapetum lucidum, exophthalmia, papilledema, roughness of coat, poor flesh, slowness of growth, muscular incoordination, humped back, diarrhea, colds, pneumonia, blindness and death. The gross and microscopic pathology observed at autopsy included a cystic condition of the pituitary, a constriction of the optic foramen and various degrees of histopathological changes in the tissues of the intestines, liver, kidney and testicle.

An observation which appears to be of considerable importance is the relationship which appeared to exist between the vitamin A content of the blood and the environmental temperature. This is illustrated by all three figures (1–3 inclusive). This relationship indicates that either vitamin A is required in greater amounts during cold weather or that more of the vitamin is oxidized by the increased metabolism required to maintain body temperature. Respiratory disturbances and diarrhea appeared to be more prevalent during periods of low blood vitamin A thus adding another complicating factor to the situation. The fact that on the higher levels of carotene intake these conditions were observed only rarely, appears to eliminate them as the cause of the decreased blood vitamin A. The logical assumption is that these conditions were brought about in the calves on the lower levels of carotene intake as a result of the low concentration of blood vitamin A or by the same conditions which produced the low blood vitamin A.

The effects of vitamin A deficiency on the intestinal mucosa would seem to act toward producing a sort of vicious circle. Under these conditions, vitamin A deficiency tends to produce an enteritis which results in a diarrhea and probably in a decreased absorption of carotene. This would tend to make the deficiency more marked and thus produce a more severe enteritis. The importance of adequate dietary vitamin A, in this respect, can be readily appreciated. Colds followed by acute bronchial pneumonia might also tend to act in a similar manner. The vitamin A deficiency probably tends to cause a decrease in the resistance of the upper respiratory tract against infection. The infection produced then tends to increase heat production by means of a fever, thus probably bringing about an increased destruction of vitamin A and a greater vitamin A deficiency. Although most of this is still theory, it may offer explanations for some of the phenomena observed.

Another observation which may be of considerable importance was the greater amount of vitamin A in relation to carotene in the blood of young calves as compared to that of older calves. This observation would indicate that nature has made it so that the young animal converts carotene to the essential vitamin A either more efficiently or more rapidly as a means of giving it increased protection during this critical period of life. It is possible that the difference in the ability to convert carotene to vitamin A may be responsible for the greater ease with which Holstein calves are raised than is the case with Guernseys. This factor may function both before and after birth.

Actual observations bear out the difference in carotene requirement indicated by blood vitamin A levels at different environmental temperatures. Calves which received around 12 micrograms of carotene per pound of body weight per day during warm weather appeared to get just about enough to prevent vitamin A deficiency symptoms. However, calves that received 20 to 23 micrograms of carotene per pound of body weight per day during the winter months have been observed to show deficiency symptoms during or immediately following periods of unusually cold weather. The effects of weather changes were very evident in the eyes of the affected calves. Further effects of temperature changes were indicated by the vitamin A and carotene values obtained on the livers.

The gross and microscopic studies made on the organs and tissues support the observation just noted. The extent and severity of these changes were almost without exception roughly proportional to the severity of the external symptoms of vitamin A deficiency observed, particularly after an animal had shown deficiency symptoms for some time. It is important to note that definite histopathological effects of vitamin A deficiency were found in animals which had been considered to be getting the minimum carotene intake. Practically all such animals would be considered normal by the average dairyman. This brings up the important fact that such cases as these on the borderline of deficiency are probably very widespread, particularly in herds in regions where the roughage is usually of poor quality. It is highly probable that such effects might result in the production of cows that would lack in vigor, in milk producing ability, and in reproductive efficiency, without the real cause being suspected. It is possible that the most detrimental effect of previous vitamin A deficiency in dairy cattle lies in such cases.

CONCLUSIONS

1. The minimum carotene requirement of dairy calves maintained at an environmental temperature of 50 to 70° F. was found to be approximately 12 micrograms per pound of body weight per day.

2. The minimum carotene requirement for growth and well-being of dairy calves appears to depend upon environmental temperature. During severe winter weather the minimum requirement may be more than twice as great as during warm weather. This increased requirement is substantiated by the observed lowering of blood carotene and blood vitamin A during cold weather.

3. Respiratory and bowel disturbances were more prevalent during periods of low blood vitamin A than when the store of blood vitamin A was more abundant.

TABLE 1

Summary of the experimental set-up employed and the results obtained with the individual calves

Calf No.	Sex	Breed	Date slaughtered	Carotene intake micrograms per lb. per day	Pathological conditions		Adequacy of carotene intake
					Gross	Microscopic	
E17	M	G	7–28–39	3.7 at 9–28 weeks of age	Papilla—atrophied Pituitary—cystic Liver—scarred Kidney—grayish yellow foci Intestine—marked thickening of mucosa Blindness	Kidney—degeneration of tubules Liver—degeneration and necrosis Intestine—inflammatory changes	Definitely deficient after 7 weeks of age
E18	M	G	8–13–39	11.8 at 11–15 weeks of age 12.7 at 16–29 weeks of age	Normal except diarrhea	Kidney—slight degeneration of some of tubular epithelium	Deficient 5–14 weeks, about on borderline thereafter
E19	F	G	7–27–39	4.7 at 10–15 weeks of age 5.1 at 16–28 weeks of age	Papilledema Lungs—consolidated in small area Heart valves—small cysts Intestinal epithelium—hyperplastic Stomach—hemorrhagic Gall bladder—walls thickened Nearly blind	Kidney—degeneration Intestine—inflammatory changes Liver—degeneration and necrosis	Deficient after 8 weeks of age
E20	M	G + H	8–14–39	10.7 at 10–15 weeks of age 11.4 at 16–29 weeks of age	None	Kidney—slight degeneration Liver—several foci of necrosis	Deficient at 7 weeks. Gradually recovered. On borderline after 13 weeks

TABLE 1—(*Continued*)

Calf No.	Sex	Breed	Date slaughtered	Carotene intake micrograms per lb. per day	Pathological conditions		Adequacy of carotene intake
					Gross	Microscopic	
E21	M	G	8–16–39	5.9 at 12–17 weeks of age 6.4 at 18–32 weeks of age	Pituitary—slightly cystic Optic nerve—moderately constricted Liver—slight scarring of parenchymatous tissue Kidney—small grayish white areas on sectioning Intestinal mucosa—mild hyperplasia Papilledema	Kidney—marked degeneration of tubules Testicle—marked degeneration of germinal epithelium Intestine—extensive hyperplastic enteritis	Deficient after 8 weeks of age
E23	F	G	8–16–39	7.1 at 10–15 weeks of age 7.6 at 16–30 weeks of age	Kidney—small grayish white areas on sectioning Intestine—moderate enteritis of subacute nature Papilledema	Kidney—slight glomerular nephritis Intestine—moderate inflammatory changes	Deficient after 7 weeks of age—slight to moderate changes
510	F	H	10–24–39	7.1 at 5–10 weeks of age 7.6 at 11–34 weeks of age	Pituitary—cystic Intestine—marked hyperplasia Optic nerve—constricted Papilledema Kidney—small grayish white foci on surface and cut section	Kidney—early changes Liver—slight degeneration Intestine—moderate inflammatory changes	Deficient after 10 weeks of age. Improved from 29 weeks until slaughtered at 34 weeks

TABLE 1—(*Continued*)

Calf No.	Sex	Breed	Date slaughtered	Carotene intake micrograms per lb. per day	Pathological conditions		Adequacy of carotene intake
					Gross	Microscopic	
513	M	H	9- 7-39	9.5 at 3–8 weeks of age 10.0 at 9–26 weeks of age	Papilledema Intestinal mucosa—very thickened and severe diarrhea Lungs—slight congestion	(Not examined)	Deficient after 9 weeks of age
514[*]	M	H	10–25–39	10.7 at 3–8 weeks of age 11.4 at 9–32 weeks of age	Papilledema	Kidney—moderate changes Liver—slight changes Intestine—slight enteritis	Borderline of deficiency 17–25 weeks. Deficient thereafter
516	M	H	10–20–39	4.7 at 2–7 weeks of age 5.1 at 8–31 weeks of age	Pituitary—cystic Optic nerve—markedly constricted Papilledema Small intestine—very hyperplastic Almost blind	Kidney—tubular degeneration and fibrosis Liver—fatty degeneration Small intestine—hyperplastic enteritis Testicle—slight degeneration	Deficient after 4 weeks of age
521	M	H	12–15–39	8.3 at 3–6 weeks of age 8.9 at 7–30 weeks of age 14.7 at 31–38 weeks of age	Pituitary—cystic Intestine—mild enteritis above iliocecal valve Papilledema	Kidney—Early degenerative and inflammatory changes Liver—marked changes Intestine—marked changes	Near borderline 14–26 weeks. Deficient thereafter

TABLE 1—(*Continued*)

Calf No.	Sex	Breed	Date slaughtered	Carotene intake micrograms per lb. per day	Pathological conditions		Adequacy of carotene intake
					Gross	Microscopic	
523	M	H	12– 3–39	6.4 at 2–25 weeks of age 10.5 at 26–32 weeks of age	Papilledema Pituitary—cystic Lungs—anterior lobes consolidated and abscessed Kidney—Small foci on cortical surface	Kidney—early degenerative and inflammatory changes Intestine—marked changes Liver—marked changes	Deficient after 12 weeks. Gradually became worse
524	M	H	1– 9–40	11.4 at 2–25 weeks of age 18.9 at 26–37 weeks of age	None	Kidney—some degeneration and cellular infiltration	On or just below borderline after 26 weeks of age (cold weather)
528	M	H	2–13–40	12.7 at 2–18 weeks of age 21.0 at 19–35 weeks of age	None	Kidney—slight degeneration and slight cellular infiltration Testes—considerable degeneration	On borderline 25–33 weeks (cold weather). Appeared O.K. when killed
529	M	H	2–12–40	16.5 at 2–16 weeks of age 27.3 at 17–33 weeks of age	None	Kidney—early changes with a few areas of fibrosis and hemorrhages in tubules	Near borderline during last several weeks (cold weather)
533	M	H	6– 4–40	10.2 at 2–6 weeks of age 16.8 at 7–21 weeks of age 18.4 at 22 weeks of age and decreased gradually to 16.8 at 39 weeks	Slight papilledema remained	Intestine—a moderate enteritis	Slight deficiency 12–33 weeks (cold weather). Appeared sufficient 34–39 weeks

TABLE 1—(*Continued*)

Calf No.	Sex	Breed	Date slaughtered	Carotene intake micrograms per lb. per day	Pathological conditions		Adequacy of carotene intake
					Gross	Microscopic	
534	M	H	5–24–40	12.7 at 2–5 weeks of age 21.0 at 6–18 weeks of age but then decreased gradually to 18.8 at 36 weeks	None	(Not examined)	Slight deficiency 24–29 weeks (cold weather). Appeared normal thereafter
537	M	H	6– 4–40	23.1 at 2–13 weeks of age and then gradually decreased to 20.5 at 33 weeks	Pituitary—slighlty cystic Eyes—exophthalmia and slight papilledema Lungs—some consolidation Heart—cystic ventricular valves	Intestine—moderate enteritis	Slightly below borderline 16–24 weeks (cold weather). Intake appeared sufficient after 25 weeks of age
538	M	H	6–10–40	23.1 at 2–14 weeks of age 18.5 at 15 weeks of age and decreased gradually to 16.8 at 32 weeks of age	Slight eye changes remain	Intestine—mild enteritis Liver—slight degeneration in few areas	Slightly deficient 17–27 weeks (cold weather). Intake appeared adequate at end
E24	M	G	6–10–40	18.9 at 3–13 weeks of age then decreased gradually to 16.8 at 34 weeks	None	(Not examined)	On borderline of deficiency 9–25 weeks (cold weather). Sufficient 27–34 weeks

TABLE 1—(*Continued*)

Calf No.	Sex	Breed	Date slaughtered	Carotene intake micrograms per lb. per day	Pathological conditions		Adequacy of carotene intake
					Gross	Microscopic	
E25	M	G	6–10–40	21.0 at 2–12 weeks of age then decreased gradually to 18.6 at 33 weeks	None	(Not examined)	Slight deficiency 17–26 weeks (cold weather). Sufficient 27–33 weeks
E30	M	G	2– 2–40	23.1 at 2–15 weeks of age	None	Kidney—some degeneration and some cellular infiltration	On borderline of deficiency
548	M	H	6–27–40	16.3 at 2 weeks of age gradually decreased to 14.9 at 20 weeks of age, then 3 weeks with none	Papilledema—developed during last 3 weeks Left eye—almost blind at end of experiment	Kidney—early changes Intestine—slight enteritis	Just about enough except slight deficiency 10–16 weeks (cold weather). Deficient at end
551	M	H	6–27–40	6.1 at 2 weeks of age decreased gradually to 5.6 at 19 weeks of age. None last 3 weeks	Papilledema Exophthalmia Blindness	Kidney—moderate changes including some degeneration of tubules and cellular infiltration	Deficient after 10 weeks and gradually became worse

4. The severity of the gross and microscopic pathology agreed very well with the degree of deficiency observed before the calves were slaughtered.

5. Histopathological studies reveal that calves receiving a carotene intake of less than 27 micrograms per pound of body weight per day may not be fully protected when subjected to average winter conditions.

6. It is highly probable that such histopathological changes exist in calves raised in regions where roughage of poor quality is fed and that these conditions may affect the productive and reproductive efficiency of the dairy cattle of such regions.

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FURTHER STUDIES ON THE USE OF BASIC DYES FOR MEASURING THE HYDROLYSIS OF FAT

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In a recent paper (1), the author has shown that hydrolysis of fat can be detected by means of a number of common basic dyes, provided that the base of the dye, which is usually fat soluble, has a different color from its salts. Hydrolysis is then indicated by the appearance of the color characteristic of the soap formed by the union of the dye base with the free fatty acid. It was also stated in that paper, that this fact can be utilized for the identification of neutral fats, fatty acids and lipoids in microscopic preparations and it was suggested that the choice of the dye to be used will probably be determined by the conditions and the object of the particular investigation.

The present study is concerned with the determination of the value and limitations of several common dyes when used as reagents in fat analysis. It includes observations on the ease with which the dye base is prepared, on the stability of that base, on the suitability of its color for observation with the eye or with the microscope, and on the way it may be used for a particular purpose.

Dyes investigated. The dyes investigated are *Nile blue, methylene blue, neutral red* and *spirit blue.* The last one was recently used and recommended by Starr (2) for the demonstration of fat hydrolysis by bacterial colonies. The brands used are as follows: Nile blue A, C.I. No. 913, and *spirit blue,* Schultz No. 521, both purchased from the National Aniline and Chemical Co., Inc.; Nile blue hydrochloride and methylene blue (rectified for blood stain), both purchased from Coleman and Bell Co., and a German made (Grübler) sample of neutral red. Other samples of dyes, of which we have no record, had been used previously.

Remarks on technique. The technique used in the present work is generally very simple. It is based principally on differential solubility of the dye salts and dye bases, and on the color and behavior of the various substances in the various solvents used. Basic and non-basic substances are separated by shaking with dilute mineral acids (3) and extraction with the proper solvent. Comparative quantitative conclusions are drawn from the depth of colors in definite volumes of solutions.

Preparation of the dye bases. In most of the present work the dye bases were prepared from 0.2 per cent solutions of the dye salts in water or, in the case of spirit blue which is insoluble in water, in 75 per cent, by volume, of

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ethyl alcohol, by precipitation with N NaOH. It takes 0.5–0.6 ml. of the alkali to precipitate the bases from 50 ml. of the above solutions of their salts. In the case of Nile blue and neutral red, precipitation is immediate; the base of spirit blue precipitates out gradually; while in the case of methylene blue, precipitation takes place only upon standing for a number of hours, but can be hastened by heating in flowing steam for a few minutes.

After filtration, the precipitates should be washed with slightly alkaline water of about pH 7.5–8.0, in order to prevent the formation of dye salts.

Purity, solubility and stability of the dye bases. The precipitates of the dye bases as prepared above, all contain a small amount of dye salts, probably on account of absorption of CO_2 and other acids from the atmosphere of the laboratory; otherwise, the precipitates of neutral red, methylene blue and spirit blue are found to consist exclusively of the bases of those dyes, and they can be completely extracted from their xylol solutions by shaking with dilute acids. However, the precipitate of the base of Nile blue is found to contain, in addition to a small amount of dye salt and of the oxazine base, a considerable amount of the oxazone which is not removed from its xylol solutions by shaking with dilute acids, and which has no tendency to form soaps with free fatty acids in which it simply dissolves with a purplish red color. The oxazone may sometimes be found in the commercial samples of Nile blue salts where it is probably formed during the manufacturing process. However, most of the recently purchased samples of the dye are free from the oxazine base and of the oxazone, but both substances can be demonstrated as soon as the dye is dissolved in water.

The dye bases are stable in the form of dry powder, but their behavior in solution requires some discussion.

1. *The base of Nile blue.* The base of Nile blue is practically insoluble in water. Its xylol solutions are stable. It dissolves readily in 75 per cent, by volume, of ethyl alcohol, in which it undergoes a series of transformations. When first prepared, the alcoholic solution is deep red, but upon standing for a few days, it gradually turns blue. Repeated studies on a 10-day-old solution led us to the conclusion that it contained a small amount of dye salt and a certain amount of the oxazone, but that the rest consisted of a base which forms, in xylol, a dark red solution with a purple fluorescence. We are inclined to believe that this base is a second form of the original oxazine base. It forms a purple blue soap with oleic acid and temporarily reverts to the original oxazine base upon the addition of a small amount of N NaOH. However, a strongly alkaline solution of the base of Nile blue in 75 per cent alcohol (25 ml. of solution + 0.5 ml. of N NaOH) gradually turns brownish green, and it can be readily demonstrated that it consists mainly of a non-basic substance soluble both in water and in xylol. In water solutions, this substance acts as an indicator turning into a light reddish brown color upon the addition of acid. In strongly acid solutions, a tan precipitate

is formed. However, regardless of the pH of the aqueous solution, shaking with xylol extracts the color from the aqueous phase and gives a red xylol layer. Both neutral butter fats and free oleic acid extract the color from the water solutions and assume a red color, although the free fatty acid shows a slightly darker shade of red.

Attempts were made to preserve the original pink form of the oxazine base by adjusting the pH of its solutions. To accomplish that purpose portions of a saturated solution of the oxazine base in 75 per cent alcohol were saturated with dibasic potassium phosphate, sodium bicarbonate or sodium borate. After standing for about two weeks in the dark in partially filled, stoppered test tubes, the portions were examined and found to be respectively blue, purple and reddish purple. Only the portion containing phosphate still contained some base. The portions containing the bicarbonate and the borate contained no base, but mostly a non-basic substance having the properties of the oxazone. In all cases a fair amount of dye salt was also present.

2. *The base of methylene blue.* The base of methylene blue is fairly soluble in water with which it forms purplish blue solutions. It readily forms a blue solution in 75 per cent, by volume, of ethyl alcohol, and a dark red solution with xylol. Those solutions are all stable. Portions of the saturated water solution, saturated with dipotassium phosphate, sodium bicarbonate and sodium borate, and allowed to stand for 5 days in the dark, showed no other xylol soluble substance than the thiazine base. The base is not present in the commercial samples of dye salt powder, nor in its freshly prepared solutions.

3. *The base of spirit blue.* The base of spirit blue is insoluble in water but it is soluble in 75 per cent, by volume, of ethyl alcohol giving a purplish blue solution. In xylol, the color of the solution seems to depend on the concentration and to vary accordingly from yellow through orange to deep red. The color of the xylol solution has a tendency to fade upon standing for reasons yet unknown to us.

When portions of the dye base solution in 75 per cent alcohol are saturated with dipotassium phosphate, sodium bicarbonate or sodium borate, and allowed to stand in the dark for five days, the portion containing the phosphate remains blue, that containing the carbonate gives a purple precipitate and a reddish supernatent liquid, while the portion containing the borate gives an orange red precipitate and a faintly pink supernatent liquid. This corroborates other observations we made indicating that the solubility of the base in 75 per cent alcohol is greatly reduced as the solution is made more alkaline. Under all the above conditions, however, no xylol soluble, non-basic substances were ever detected by us. The free base is not present in the commercial sample of the dye salt used, but it is present in traces in freshly prepared solutions of the dye salt in 75 per cent alcohol.

4. *The base of neutral red.* The base of neutral red is slightly soluble

in water with an orange yellow color. It is very soluble in 75 per cent, by volume, of ethyl alcohol, giving a brownish yellow solution, and it gives with xylol an orange yellow solution with a green fluorescence. It is quite stable in all its solutions. Saturation of its alcoholic solutions with dipotassium phosphate, sodium bicarbonate or sodium borate produces no important changes. The free base is not present in the commercial samples of the dye salt used, but it appears as soon as the salt is dissolved in water, probably because of hydrolysis.

DISCUSSION

It is obvious from the above study that Nile blue, the first basic dye to be used for the analysis of fat, is highly unstable and, therefore, unsuitable for that purpose, although it owes its discovery as a fat dye to that instability and to the weakness of its base. Of the three remaining dyes, selection must be made according to the requirements of the specific problem, with due consideration to solubility and contrast. In our own work, we have selected neutral red base as a general reagent in the analysis of fat, and in the specific problem of detecting the hydrolysis of fat by colonies of microorganisms. We have also adapted it for the quantitative determination of fatty acids in edible fats, including the problem of determining the quality of butter. The ease with which the base of neutral red is prepared and its stability under different conditions recommend it for consideration by the bacteriologist as well as by the chemist. Microscopically, the base is also usable in most cases. However, on account of the somewhat poor contrast between its color and that of its soaps, we have been in the habit of supplementing it with some of the other bases in the study of very minute fat droplets such as those that occur in the bacterial cell. The use of dye bases in bacterial cytology will be the subject of a future investigation.

SUMMARY

A study is made of the free bases of Nile blue, methylene blue, spirit blue and neutral red, especially with respect to preparation, solubility, stability and contrast. The base of Nile blue is found to be highly unstable and unsuitable for use in fat analysis. Of the four dyes studied, the base of neutral red is recommended for general use. When excessive contrast is desirable, as in the microscopic study of very minute fat droplets, the use of neutral red base may be supplemented with some other dye base. Adaptation of the use of dye bases for determining the quality of edible fats has yielded promising results and is under further investigation.

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A COLORIMETRIC METHOD FOR ESTIMATING THE QUALITY OF BUTTER.—A PRELIMINARY REPORT

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In presenting a test for the quality of butter, the authors realize that the quality of butter is a resultant of a number of characteristics some of which are not measurable by physical or chemical means. Therefore, the definitive evaluation of the quality of butter must perforce remain a matter of art and judgment. However, dealing with a system consisting principally of fat, it is to be expected that many of the changes that take place in butter would involve the fat component, and the present test is a quick and simple way of measuring the degree of hydrolysis of the fat of the butter.

In a recent paper, Knaysi (2) has shown that the free bases of basic dyes are usually soluble in neutral fat, and that the color of certain dye bases is different from that of their soaps. Therefore, if the proper dye base be dissolved in neutral fat, hydrolysis is immediately indicated by a shift in the color of the fat solution toward that of the soap, and the extent of the shift is a measure of the degree of hydrolysis. The reaction is that of simple neutralization:

Dye base + Free fatty acid = Dye soap + water

It is, therefore, to be expected, and it can be readily demonstrated, that a direct, simple proportion exists between the shift in the color of the fat solution and the degree of hydrolysis.

TECHNIQUE

In this work, we made use of the base of neutral red which has been shown by Knaysi (3) to be quite stable and easy to prepare. It can be prepared from the aqueous solution of the dye salt by precipitation with sodium hydroxide. About 0.5 ml. of N NaOH are sufficient for complete precipitation from 50 ml. of solution containing 0.2 gm. of neutral red. The precipitate is filtered and washed with slightly alkaline distilled water of pH 7.5–8.0. It is then dried and kept for use. It is only slightly soluble in water but dissolves readily in xylol and in neutral fat. Its xylol solution is orange yellow with a green fluorescence.

In order to test the degree of hydrolysis of milkfat, a few grams of butter are placed in a clean test tube and melted at 60° to 70° C. The tubes are allowed to stand in the water bath until most of the coarse particles of casein and other non-fatty material have settled to the bottom of the tube. That

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usually takes about 15 minutes. One ml. is then measured into another clean test tube and dissolved in 3 ml. of a saturated solution of the dye base in xylol and the color of the resulting solution compared with standards containing known quantities of oleic acid. It is important that the tube containing the milkfat and those containing the standards be approximately of the same diameter.

The standards are prepared as follows: A series of 10 clean test tubes of approximately the same diameter are placed, in order, in a rack or a block. They are labelled, respectively, 0, 2, 3, 5, 7, 10, 12, 15, 20 and 30. To each tube, oleic acid solution in xylol (C.P.), pure xylol and saturated solution of neutral red base in xylol are added according to table 1. The total volume in each tube is then 4 ml., and the color of each standard is more reddish the greater the amount of oleic acid it contains. The standards are stable if tightly stoppered.

TABLE 1

Preparation of standards

No. of standard	1% oleic acid*	10% oleic acid*	Pure xylol	Saturated xylol solution of dye base
	ml.	*ml.*	*ml.*	*ml.*
0	0.00	0.00	1.00	3.0
2	0.20	0.00	0.80	3.0
3	0.30	0.00	0.70	3.0
5	0.50	0.00	0.50	3.0
7	0.70	0.00	0.30	3.0
10	1.00	0.00	0.00	3.0
12	0.00	0.12	0.88	3.0
15	0.00	0.15	0.85	3.0
20	0.00	0.20	0.80	3.0
30	0.00	0.30	0.70	3.0

* In xylol, by volume.

Comparison with the standards can often be made within a few minutes and bad samples can be detected immediately. However, enough non-fatty material is frequently carried in the sample to cause turbidity in the tube containing the butter sample, and the exact comparison with the standards is possible only after standing for several hours or centrifuging with a hand centrifuge for a few minutes.

DATA AND DISCUSSION

During the course of this work, the test was applied to more than a hundred samples of experimental or commercial butter. The experimental samples were either prepared for the present purpose or had been prepared for other investigations. The commercial samples were mostly bought from stores in this vicinity and represent a cross section of the butter consumed in this section. Specifications about those samples together with the evaluation of their quality by the present test or by the usual score can be found in tables 2 to 4.

1. *Effect of acid or salt.* Nine samples of butter, A to I, were churned on Feb. 27. A to D were made from sweet cream; C and D were salted. E to I were unsalted and made from pasteurized cream to which starter was added; E was churned immediately after the addition of the starter, while F to I were churned after incubation of the cream for increasing periods at 70° F. to allow the development of acidity. All the samples were judged on the same day and were given a score of 95. They were placed in a cold room (0° F.) till the next day when samples E–I were tested by the present method. Samples A–D were tested on March 1. The results are summarized in table 2 and indicate that salted butter has a slightly higher fatty acid content than sweet butter made from the same cream. Whether the effect of the salt is direct or indirect we are not yet able to say. The results also show that when the cream develops high acidity, the test indicates an increase in the free fatty acid content of the butter. That the effect is not due to the lactic or acetic acids of the cream can be concluded from the fact that addition of the latter two acids has no other effect than the extraction of a part or all of the base from the xylol phase, depending on the relative concentration. It is probably due partly to holding at 70° F. and partly to promotion of fat hydrolysis by the developed acidity. A slight hydrolysis of milkfat from acid cream has also been observed by Herrington (1).

TABLE 2

Effect of acids and salt

Sample	Type	Equivalent standard	Score
A	Sweet cream. Unsalted	1	95
B	Sweet cream. Unsalted	1	95
C	Sweet cream. Salted	3	95
D	Sweet cream. Salted	3	95
E	Starter added	2	95
F	Starter + 2½ hrs. at 70° F.	2	95
G	Starter + 3½ hrs. at 70° F.	2	95
H	Starter + 4½ hrs. at 70° F.	3	95
I	Starter + 5 hrs. at 70° F.	5	95

2. *Effect of starter distillates.* The addition of starter distillates to butter made from sweet cream is practiced by certain creameries. It was therefore opportune to find out whether such a practice would have any effect on the results of the present test. For that purpose a certain amount of pasteurized, high grade sweet cream was churned and the butter divided into seven portions, J to P. Sample J was the control. Portions K to M received respectively 1, 2 and 3 drops of Verley S.D.C. per 20 grams, and portions N to P received respectively equal amounts of Hansen's distillate.

The next day, all portions were judged and tested by the present method. Table 3 shows that the addition of those distillates in the proportions indicated has no significant influence on the results of the test. It is worth noting, however, that portion P was given a lower score because of a detrimental effect of the quantity of distillate added on the flavor, a defect which was not reflected by any hydrolytic change in the fat, and which our test could not bring out.

TABLE 3

Effect of starter distillates

Sample	Starter distillate and amount	Equivalent standard	Score
J	None	0	95
K	1 drop of Verley SDC/20 gms.	0	95
L	2 drops of Verley SDC/20 gms.	0	95
M	3 drops of Verley SDC/20 gms.	1	95
N	1 drop of Hansen's dist./20 gms.	0	95
O	2 drops of Hansen's dist./20 gms.	0	95
P	3 drops of Hansen's dist./20 gms.	0	94

3. *Effect of the method of melting the butter sample.* Two portions of each of a series of butter samples were placed in two test tubes. In each case one portion was melted in flowing steam for 5 minutes and then placed for ten more minutes in a warm place to allow settling of the coarse non-fatty particles. The second portion was placed for fifteen minutes in a water bath at 65° C. Table 4 shows that there is usually no significant difference

TABLE 4

Effect of the method of melting the butter sample

Sample	Description	Equivalent 65° C.	Standard steam	Score	Flavor
1	Recently purchased	1	1	92	Old cream
2	Recently purchased	2	2	90	Old cream
3	Recently purchased	3	3	89	Old cream
4	Recently purchased	3.5	3.5	89	Slightly strong
5	Recently purchased	2	2	91	Old cream
6	Recently purchased	1	1	93	
7	Recently purchased	2	1	93	
8	Stored at 0° F.	2	2	88	Old cream. Storage
9	Stored at 0° F.	2	2	88	Old cream. Storage
10	Stored at 0° F.	2.5	3	89.5	Old cream. Storage
11	Stored at 0° F.	1	1	92	Storage
12	Stored at 0° F.	5	5	83	Fishy
13	Stored at 0° F.	2.5	2.5	87	Storage
14	Stored at 0° F.	2	2	90	Storage
16	Stored at 0° F. (15 yrs. old)	8	5	83	Tallowy
21	Stored at 0° F.	30	30	83	Bitter-rancid
28	Stored at 0° F.	2.5	2	89	Old cream. Storage
30	Stored at 0° F.	2.5	2	89	Old butter flavor
31	Stored at 0° F.	2	2	89	Old butter flavor
32	Stored at 0° F.	1	1	89	Old butter flavor

in the results of the test, although we often observed a faintly less reddish tinge in the portion melted in the flowing steam, probably due to a small loss of some of the volatile fatty acids.

CONCLUSIONS

An analysis of the data presented in tables 2–4, together with other similar data which we do not find it necessary to report, justifies our introductory remarks that no perfect agreement should be expected between the quality of butter as estimated by the present method and by the score of the expert.

However, it may be concluded that butter which, by the present test, corresponds to a standard greater than 5 is of poor or bad quality, and that corresponding to a standard 10 must be considered unfit for consumption. When the test is below 5, *i.e.*, when the free fatty acid content of the butter is low, the butter may still be given a low score on the basis of other factors, the estimation of which is largely a matter of art and judgment. In the lower range, a test of 2 or below indicates, almost always, butter of fair, good or excellent quality.

We feel, therefore, that the present test, which is a very simple and accurate measure of free fatty acids, should prove to be of value in detecting bad samples and the majority of fair or good samples of butter, and to be of help to the expert by giving more precision to his judgment regarding the actual state and the probable keeping quality of a given product.

SUMMARY

A simple method of estimating the quality of butter is described. It consists in dissolving 1 ml. of the melted milkfat in chemically pure xylol saturated with the base of neutral red, and comparing the color with standards containing known quantities of oleic acid. The preparation of the base of neutral red is described.

The test, which is a measure of the degree of hydrolysis of the butter fat, is found to be of value in quickly detecting bad samples and the majority of fair or good samples of butter, and in adding precision to the judgment of the expert.

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THE NUTRITION OF CALVES; A REVIEW

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> *"But most of all it will be worth while for him to realize how small and dim is the present light of knowledge in the vast field of ignorance of nutritional questions."*—E. B. Meigs, 1923.

EARLY HISTORY

Early students of nutrition and physiology were usually interested in the comparative aspects of their subject. Therefore, the calf, sheep and cow were studied as well as the dog and fowl. From the beginning the general interests fell in two classes, physiologists were concerned with the fate of food in the gastrointestinal tract, agricultural workers were interested in rearing good calves with a minimum wastage of whole milk.

As early as 1777 Stevens (242) at Edinburgh made some initial studies of digestion in ruminants by feeding sheep perforated silver spheres and noting the rate at which these lost their contents while in the rumen. Even earlier, in 1752, Reaumur (208) had suspended tubes of hay and green grass in the rumens of sheep but had been disappointed in the lack of digestion after fourteen hours. In 1768 Batigne (14) criticized these studies because the feeds were not chewed. Spallanzani (240) corrected this error and reported in 1784 that chewed feeds were digested by oxen when fed in perforated tubes but unchewed ones were not.

Spallanzani made a number of observations upon herbivora. He noted that tubes took about twenty-five hours to pass through the ox. He felt that rumination was analagous to trituration in birds. He knew that herbivorous animals could subsist upon flesh if other foods failed. He also was among the first to believe that the rennin of the calf's stomach acted because of its enzyme properties rather than because it was an acid.

By the time the great work of Tiedemann and Gmelin (251) upon digestion appeared in 1826, much was known about digestion in several species. These authors found that milk fed to calves passed directly into the fourth stomach. They attempted to analyze different parts of the gastrointestinal tract but made little progress because of crude methods. After feeding hay to cows, these authors found various gases produced in the rumen. They were able to use lead acetate and get a test for hydrogen sulfide. These authors also knew the true stomach of the ruminant contained acid. They also obtained a red color from testing the contents of the upper small intestine with chlorine. This was probably the earliest evidence for the production of tryptophane through the digestion of protein. These authors tied off the pancreas in a sheep but produced no marked results.

These same authors fed colored water to ruminants and observed that this water distributed itself in all four stomachs. In the rumen they also recognized CO_2, NH_4 and butyric acid. They believed the rumen contents were slightly alkaline. The muscles concerned and the mechanism of rumination had been well studied before the time of Tiedemann and Gmelin but they reviewed the knowledge of their time. For the determination of hydrogen ion concentration the common indicators of 1826 were tinctures of mauve, litmus and violets.

These authors found the large intestinal contents to be neutral or slightly alkaline in herbivora fed hay but in the case of calves fed milk they claimed these contents were acid. By 1826 they were already familiar with the major processes of digestion. They even recognized the functions of bile in preventing putrefaction and in the emulsification of fats.

About a hundred years ago the use of fistulae for the study of digestion became common. Human accidents had paved the way. In 1842 Bassow (13) and also Blondlot (29) prepared gastric fistulae in dogs. These techniques were soon applied to herbivora by P. Flourens (80) and are described in his work of 1844. This field of work never became popular and even today has been insufficiently used in studying herbivorous species.

In general the nineteenth century was quite sterile in developing improved methods for feeding calves. The few methods of rearing calves upon milk substitutes seemed to have originated early in the century. Boussingault (30) in 1845 discusses some of his experiments in feeding calves upon hay tea and upon suspensions of "oil cake," probably linseed meal. He noted that some dairymen advocated teaching the calf to drink immediately after birth. In the best Swiss dairies of a hundred years ago he notes the calves were fed liberally upon whole milk for six weeks. At four weeks of age they were given chopped hay and some roots. After about two months the calves were fed 8 to 9 pounds of hay daily for the first year and about double this during the second.

Under such conditions Boussingault found the calves drank fourteen to eighteen pounds of milk daily for the first six weeks. The calves of Boussingault's "Horned Cattle" averaged 96 pounds at birth. During the suckling period the calves gained over two pounds per day. At this early date they were experimenting with "hay tea," an infusion of hay in warm water. They obtained fair results with it but Boussingault felt it was a questionable procedure. After the suckling period the average daily gain was 1.5 pounds.

Among the early substitutes for whole milk in calf feeding were skimmed milk and linseed oil. Carbohydrates were also tried. Liebig (143) tried his hand at devising a calf gruel. He used a mixture of wheat flour boiled in water and then treated with ground malt and $KHCO_3$. This was supplemented with whole milk. Kellner (127) described various modifications of such gruels. These mixtures were all low in fat-soluble vitamins and it is surprising that calves could grow upon them.

The common ingredients of calf meals before the year 1900 were wheat flour, flaxseed meal, coconut meal, ground beans and peas, cottonseed meal, tapioca, starch, and salt. Some listed such ingredients as rice polish and fenugreek which are interesting today as sources of water-soluble vitamins and choline. In the early bulletins of Morse (186) and Lindsey (146) are lists of ingredients used in early gruels.

A good review of the better practices in calf rearing by early Americans is given by Stewart (243) in 1883. He advocated feeding a month or two upon whole milk and then changing to flaxseed or linseed meal. He trained his calves to drink when they were a week old. He used a pint of flaxseed and one of linseed oil meal boiled with 10 quarts of water. This was mixed with one or two parts of skimmilk and fed warm. If the calves tended to scour he gave them a quart of coarse wheat flour called "canel" for a meal or two.

Stewart was an enthusiast for flaxseed and claimed calves could be reared by the above system as well as by feeding milk. He preferred old process linseed meal, since it contained 10 per cent of fat in contrast to the new process with only 2.5 per cent, if he could not obtain flaxseed. Today we wonder that the calves did not die of vitamin A deficiency. The calves may have been able to build a slight reserve while fed whole milk and they were given hay or green grass as early as they would eat it.

Stewart devoted several pages of his book to whey feeding. He advocated oil meal as a supplement also, in this case, stressing that this meal was 28 per cent protein, 10 per cent oil and 8 per cent ash. Feeding whey alone provéd quite injurious. He reviewed an experiment at the Elgin Creamery, Illinois. In 1876 Wanzer reared 120 calves upon whey supplemented with oil meal, oats and bran. This operation proved profitable when the calves were sold at seven months of age. One still wonders how such calves evaded rickets and vitamin A deficiency.

Stewart also fed some of his calves hay tea, linseed meal and wheat middlings. These calves were started at 30 days of age and fed the diet for two months.

The number of experiments with calves during the nineteenth century, which were outstanding as permanent contributions, was very limited. One of the greatest of these was the determination of the chemical composition of the entire carcasses of animals by Lawes and Guilbert (140, 141). These studies were made from 1858 to 1861. Some of these values still serve for reference.

A second classical experiment was that of Franz von Soxhlet (239) who is best known for introducing sterilized milk in infant feeding in 1886. About 10 years before this, at the Vienna experiment station, he first determined the rate of conversion of milk into the body tissues of calves. This early work was extended (1903) in the early part of the present century.

Another contribution of great merit was that of Lehmann (142). He attempted to answer these questions: (a) Could the calf get adequate bone-building materials from the feeds commonly used? (b) Could calves digest earth phosphate if it were fed as a fine powder? Lehmann ran chemical balance studies upon his calves. His paper gives a drawing of the bags used for collecting excreta. For 14 days he fed a calf daily 500 grams of barley, 500 grams of rapeseed meal, 2000 grams of timothy and 10 kg. of milk. He then collected urine and feces for two days after which he supplemented the diet for two days with 12.8 grams of calcium phosphate daily and continued the collections for two more days. These short collection periods were justly criticized by later workers.

However, Lehmann concluded calves were getting too little calcium but enough magnesium from the usual diet. Furthermore, he recognized hay as the chief source of calcium among feedstuffs. Finally, he decided about half of the calcium phosphate was retained by the growing body of the calf.

This mineral balance of Lehmann's was stimulated by the famous pigeon feeding trials of Chossat (39) made in 1843. Chossat found the bones of pigeons became very poor in ash if they were fed upon wheat without calcium supplements.

More extensive mineral balances with calves were run by Weiske (264). He found little evidence that calves could utilize calcium phosphate. While Weiske criticized Lehmann's experiments severely he really made a lesser contribution himself.

Another experiment of indirect interest to nutrition students was that of the Earl of Spencer (60). He determined the gestation period of cows and found a range of 220 to 313 days with a mean of 284. He was never able to rear a calf if this period was shorter than 242 days.

In the light of the modern extensive feeding of cod-liver oil to farm animals, the first early report by Pollock (202) is of some interest although it is hardly an epoch-making contribution. Pollock fed sheep, swine and bullocks cod-liver oil in relatively large amounts. At two high levels he injured his swine and produced the yellow fat frequently described in modern literature after muscle degeneration has been produced by cod-liver oil feeding. He also found some injury to sheep, but none to his bullocks.

PRENATAL DEVELOPMENT AND POSTNATAL MORTALITY

Everyone recognizes the great variability in the ruggedness of calves at birth. In part this is undoubtedly due to the diet of the mother and in part to the numerous other rather poorly defined variables. Little is known about the effect of diet during the gestation period but it is undoubtedly of tremendous importance. To a certain extent the body of the mother draws upon its own resources to insure the development of the foetus. In poor areas it is common observation that good calves may be born from cows that have been poorly fed and housed. There must be a limit, however, beyond which the body stores of the mother cannot be depleted and then the young must die to preserve the adult.

The plane of nutrition of the dam has little influence on the composition of the calf if the lowest plane fed is adequate (Haigh *et al.*, 98). The calf suffers only when the lowest plane is borderline or inadequate.

This delicate balance is well illustrated in the case of iodine deficiencies where the calf may be born dead as described by various workers in the iodine deficient areas. With a little more iodine in the diet of the cow the calf may be quite normal at birth but the thyroid may be enlarged. This condition has been observed in the neighborhood of Ithaca where the iodine level of feedstuffs is slightly subnormal. In the case of iodine deficiency we are dealing with a single inorganic element in the diet. If this element is adequate in the diet of the cow there is little question about the passage through the placenta and its availability to the foetus.

The failure of the foetus to store reserves of such substances as fat-soluble vitamins is a problem very different in nature. The calf, the pig, the pup, the rat and the human infant all have a very low reserve of vitamin A stored in the liver at the time of birth (McCay 167). It is likely that the store of vitamin E is also low in vertebrates at birth although the rat is the only species that has been studied to date (Mason 153). For some unknown reason the placenta permits only limited amounts of fat-soluble vitamins to pass. Possibly there is also increased oxidation of the fat-soluble vitamins in the foetal liver due to the rich stores of iron and copper that are assembled prior to birth. The fat-soluble vitamin A tends to be lower in foetal blood than in that of the mother. Just the reverse is true for vitamin C. This vitamin tends to be higher in foetal blood and also present in relatively large amounts in the placenta. Whether or not this is one reason animals tend to eat the placenta is unknown.

The calf is born into the world with a liver relatively poor in vitamin A but rich in iron and copper. It is likely that the store of vitamin E is also low. Vitamin A is essential for the growth and vision of the young calf. Vitamin E is probably essential for prevention of degeneration of the muscles although the evidence for this rests largely upon experiments with rabbits, guinea pigs, rats, ducks, sheep and goats.

Colostrum and early nutrition of the calf. Since very early times the importance of colostrum in the nutrition of animals during the first few days and even weeks of life has been appreciated. Only during the past half century, however, have a few of the unique properties of colostrum been appreciated.

The first clear-cut evidence that colostrum was an important factor in the immunization of young animals originated in the attempts of Ehrlich (68) to transmit immunity by inheritance. Before the time of Ehrlich it was known that the young might be immune to certain diseases if the mother had acquired such immunity. Ehrlich immunized mice against the toxic proteins, ricin and albin. He then removed the young at birth from immunized to normal mothers. These young had no immunity to the poisons, but the mice allowed to suckle immune mothers had very limited immunity. This indicated that the immunity had been transferred primarily through the mammary gland with some little transfer through the placenta. In this excellent paper Ehrlich also called attention to the dilution of the immune sera in the body of the young mouse due to the rapid growth during the first days of life.

In 1907 Hohlfield (109) made limited studies with pups, kids, and young guinea pigs. In the case of the kids he found better growth during early life if they were fed colostrum.

To extend Ehrlich's observations, Famulener (74) immunized goats with sheep corpuscles before the kids were born. In order to prevent suckling before he could take blood samples, the teats were sealed with collodion. He found immunity was acquired chiefly by suckling colostrum during the first day. The colostrum tested was 2 to 3 times as rich in antibodies as the serum of the mother. By this date the colostrum was known to be rich in globulin and to contain serum proteins and it was clear that the permeability of the intestine of the newly born was different from that of older animals since only newly born seemed able to absorb antibodies when taken by mouth.

In a long series of experiments starting about 1914 but operating on a small scale, Williams (269) showed that young calves may be rather seriously infected well before birth. He made a number of studies rearing calves upon boiled milk. He found even the meconium was frequently full of bacteria due to the ingestion of the amniotic fluid by the calf. The nutrition of the cow undoubtedly influences the extent of these infections but no one has presented clear evidence to date.

About 1920 a new series of experiments were started that showed the importance of colostrum in the nutrition of the calf. Howe (110) while studying the fat metabolism of calves found they passed meconium readily without colostrum but there was some delay later in defecation. He also found the feces were rich in coagulable protein. Smith and Little (238) found much albumin in the urine of the calf during the first three days of life. Both albumin and globulin appeared in the urine if the calf consumed either colostrum or serum. The relation of this to prevention of infected kidneys needs study. The feces were about one-third dry matter and ten to fifteen per cent of this dry matter was fat and soaps. The calves seemed able to digest but not absorb the fats. Howe (111) also found that the blood of the calf contained neither euglobulin nor pseudoglobulin I at birth, but if calves were allowed colostrum these fractions appeared in the blood stream in the course of a couple of days.

In 1922 new emphasis to the importance of colostrum was given by a series of researches. Little and Orcutt (147) found colostrum provided the agglutinins for *B. abortus* found in the blood of calves. Neither the foetal blood nor that of unfed calves contained these agglutinins.

In this same year Smith and Little (238) called attention to the neglect of most textbooks in stressing the importance of colostrum in the early nutrition of the calf. They believed that the high mortality among calves that failed to get colostrum was due to the loss of protection against a number of organisms. In newly born calves they observed frequently the occurrence of minute hemorrhages in the kidneys and small intestines. Without colostrum the kidneys, spleen and liver were readily invaded by *B. coli.* Other invaders were also common and they felt the variety of pathogens made it impossible to protect calves by inoculation against specific organisms. The colostrum seemed to be the effective protective agent.

Orcutt and Howe (195) found milk would not produce the globulin fractions in blood even 21 hours after feeding but colostrum would do so in 3 hours. The rise in agglutinins and globulins was parallel.

Ragsdale and Brody (205) reviewed the chemistry of colostrum and noted, contrary to some of the earlier claims, that the content of salt and fat was low. Casein was about the same as in milk but the level of globulin was twice as high. Whereas milk contained 0.03 per cent of globulin, colostrum had 6 to 12 per cent. They considered the possibility of replacing colostrum in the diet by a mixture of milk and blood serum. In order to sterilize colostrum they found it could be heated on the water bath at 140° F. for 20 minutes. This destroyed the tubercle bacillus but did not coagulate the colostrum unless the heating was continued for 3 hours. They found colostrum could not be heated much above this temperature without destruction of the immune body.

Nelson (189) reviewed the literature bearing upon the relation between colostrum and immunity in the young calf. In his experiments he found normal cow serum tended to destroy *B. coli* and this serum was developed ultimately whether or not the calf was fed colostrum. He recognized, however, that the colostrum did contain anti-coli agglutinins and that the calf that consumed colostrum gained protection that was not afforded the calf deprived of it. Herman (105) noted that studies at Missouri show that 32 per cent of dairy calves die unless they receive colostrum.

Sato *et al.* (221) called attention to the great variability in the chemical composition of colostrum. They gave many analyses. The range of values was the following: Solids 12–27 per cent, casein 3–6, albumin 0.3–12, fat 1–13, lactose 1–5, ash 0.6–1.0 per cent. This great variability suggests important future researches to establish optimum concentrations in the colostrum for the wellbeing of the calf. No one has explored the possibility of modifying this colostrum by means of the diet of the cow during the late weeks of gestation.

The literature in this field has been reviewed by Gamble *et al.* (83). They have further explored the possibility of using mixtures of sera and milk as substitutes for colostrum in feeding foals and lambs. The substitutions were only partly satisfactory. The best results were obtained by using blood sera of the species to be fed. With the new improvements in drying blood, developed as a result of the war, still greater advances may be expected in preparing fractions of blood for feeding very young animals.

The importance of colostrum as a source of fat soluble vitamins has been recognized in recent years. Palmer (198) noted quite early that colostrum might be a source of carotene. In 1932 Dann (45) recognized that young rats were born with a poor supply of vitamin A and suggested the colostrum might have importance in providing an early supply. The colostrum of the cow was known to be 10 to a hundred times as rich in this fat soluble factor as ordinary milk.

In experiments that are unpublished we have found that young swine also are born with a very low store of A. Under normal conditions this tends to remain low for some days and it cannot be much increased by feeding young swine additional carotene. Under normal conditions the colostrum and milk of the sow probably provide enough vitamin A and an additional feeding of carotene may be destroyed. When the colostrum is low in vitamin A, the young animal might profit considerably from some special supplement fed during the first few days of life. Possibly this would be a profitable general practice (Phillips 201).

Stewart and McCallum (244) determined the vitamin A colorimetrically in colostrum, mostly from Ayrshire cows. They found it fell quickly from the first day after calving. In the 3–4 days the values were normal for milk. The colostrum varied in vitamin A from 85 to 920 international units. They obtained no good evidence that the level was related to the age of the cow, the date of calving, the breed or even the feed, although they did not explore the last possibility very extensively. They did find that lower values usually occurred in cows that had had short dry periods. They suggest that the "A" storage of the liver may be an important factor.

We have stressed earlier the possibility that the fat soluble vitamins of the colostrum may be of great importance due to the deficiency of body stores of vertebrates at the time of birth. At the present time there is special need for determining the level of the other fat soluble vitamins in the colostrum. Vitamins E and K may be of great importance in the development of the muscles and control of the coagulability of the blood.

Moore and Hallman (181) have called attention to the similarity between the white spotted kidneys found in calves fed a diet low in vitamin A and those calves deprived of colostrum.

Garrett and Overman (85) have measured the rate of decline in the mineral composition of colostrum. They found most of the elements were high and fell during the early hours of lactation. Potassium was low at the start and gradually increased. These authors provide a good review of the literature. McHargue (174) found the colostrum had 5 p.p.m. of copper on a dry basis. He also found a young calf at birth had 908 p.p.m. of copper in its liver, while one five days old had only 400.

To emphasize the importance of further studies on the nutrition of the mother during pregnancy, and any other factors that may influence the prenatal development of the calf and the condition of the calf at birth, Slack and Harrison (237) made a statistical study of the Cornell University dairy herd in 1939. We have brought these data up to 1942. In the ten-year period from 1932 to 1942, the total number of conceptions has varied from 95 in 1932 to 172 in 1940. The Cornell University herd has been free from tuberculosis and negative to the abortion blood test during this period. All the calves born prematurely, born dead, or those which died at birth because they were too weak to be raised, were listed together. The lowest number of such calves was 11 out of 137 conceptions in 1938 or 8 per cent. The largest number of conceived calves that could not be raised was 29 out of 172 conceptions in 1940 or 17 per cent. The total number of conceived calves in the 10 years that could not be raised was 169 out of 1352 conceptions or 12 per cent. The causes of these failures of the foeti to develop into strong calves are not known. It is our opinion that the loss is greater than is necessary and that some of it may be stopped through research into the nutrition of the dams as affecting the strength of the foeti.

DISEASES AND MORTALITY OF CALVES

From the very limited data published from university herds it is probable that calf losses during early life are severe. With a true appreciation of these losses it is possible that they may be materially checked both by better management and better feeding of the dam during the gestation period.

The following table was published by Ragsdale *et al.* (204) in 1926 from Missouri.

TABLE 1

Mortality of calves

Breed	Total pregnancies	Aborted		Dead at birth		Death after birth	
		No.	Percentage	No.	Percentage	No.	Percentage
Holstein	263	16	6.1	17	6.46	5	2.2
Jersey	551	70	12.7	44	7.98	36	8.3
Ayrshire	89	7	7.9	8	8.98	2	2.7
Shorthorn	40	2	5.0	6	15.00	1	2.5
Total	943	95	10.1	75	7.95	44	5.7

TABLE 2

Mortality distribution by sex

Sex	Pregnancies	Dead at birth		Mortality after birth	
		No.	Per cent	No.	Per cent
Males	413	32	8.0	16	4.2
Females	428	42	9.8	20	5.2

Jordan (124) studied the calf losses on twenty-six farms in Ayrshire. About 25 per cent of the calves born in the spring were lost and about 8 per cent in the autumn. The heavy losses were between the months of February and April. Jordan suggests that this loss is a reflection of the low vitamin level of the ration during late winter and early spring. He believes that fresh grass corrects this deficiency. His hypothesis is subject to experiment but no one has performed it. Upon inquiry of Prof. W. L. Williams of Cornell University concerning relative losses in New York State and Ayrshire, he estimated that the American losses were probably greater.

Wing (272) has provided a few data covering his experience of nearly forty years. From 644 calves, 61 died under three months, 49 were born dead and 71 were aborted. Thus 28 per cent of the calves were lost up until the change of management in 1928. This illustrates the seriousness of the losses on American farms.

TABLE 3

Distribution of 73 deaths by ages

Of a mortality of 73 calves

39 died at birth
5 died 1st day
4 died 2nd day
1 died 3rd day
7 died 3rd to 7th day
1 died 7th to 14th day
4 died 14th to 28th day
4 died 28th to 56th day
8 died 60th to 180th day

In the study made by Slack and Harrison (237), the losses in the rearing of calves at an early age were also tabulated. In the Cornell University herd in the ten-year period from 1932 to 1942, the number of calves born in good condition that soon died was not as great as the number of those conceived that did not start life at all. From page 603 you will remember that the percentage of conceptions that did not start life was 12 per cent. The lowest number of calves being raised that died in any one year was 5 in 1941, out of 166 conceptions or 3 per cent. That is an excellent record and is as low as can be expected from such a number of conceptions. The largest number lost was 23 in 1934 from 126 conceptions or 18 per cent. In 1935, 21 calves died at an early age from 109 conceptions, two less than in 1934 but a higher percentage of the conceptions, 19 per cent. Thus we see that in a large herd these losses may run from 3 to 19 per cent with an average over the ten years of 11 per cent. In those years a total of 144 calves died that were being raised from 1352 conceptions. Adding together the 12 per cent that died at or before birth and this 11 per cent, we find that 23 per cent of the calves that were conceived were not raised. This is altogether too great a loss. However, in the opinion of the authors it is a correct picture of the condition on large American farms in 1942.

Nothing is offered in the literature that will give any leads toward methods or practices that will decrease the deaths at birth and the prenatal deaths, but the losses among the well born calves that die at an early age can be overcome. This is shown in the above study in that these losses were cut to 3 per cent of 166 conceptions in 1941.

Well born calves usually die from digestive disturbances that precede calf pneumonia. The pneumonia is fatal, but is secondary. In their study Slack and Harrison (237) found that these losses were reduced by correct methods of housing.

Harrison had observed that in small herds calfhood pneumonia did not offer a serious problem. He wondered if this disease was associated with larger herds, larger groups of calves, and the continuous introduction of newly born calves among those being raised.

In October 1937 small groups of calves, not more than 8, were segregated into small barns. Each unit is separate from the others. The barns are well insulated. Each calf has a separate pen, the barn is unheated, and each group is kept by itself for at least 12 weeks. Only in the severest weather is it necessary to furnish a little heat by an ordinary electric heater to keep the calves from freezing. Conditions of management, feeding, personnel have been practically the same for the ten-year period.

In the six calendar years 1932 to 1937, 109 of the 515 calves strong enough to be raised died from pneumonia or similar diseases. This is 20 per cent. After the small barn segregation plan was put into effect this loss dropped to 6.7 per cent. In the years 1938–1941 inclusive, only 35 out of 522 well born calves or 6.7 per cent were lost. The small unit segregation was a major factor in this success because all the other factors remained the same.

Phillips *et al.* (201) have reduced this loss from scours and pneumonia by feeding large doses of vitamins A, D, thiamin, riboflavin, nicotinic acid, pantothenic acid, and choline to calves from birth to 4 weeks. This work needs to be duplicated to determine if optimum doses of A and D for the first four weeks will not be just as effective. We believe that a combination of Harrison's segregation plan and the feeding of large doses of A and D will reduce the losses of young calves to a minimum. Further investigations along the lines suggested by Phillips will determine which factors of the B complex should be fed.

THE NORMAL CALF

The growth and development of the healthy calf in terms of values subject to measurement is of importance to the nutrition student. Without standards for the normal, deviations cannot be appreciated. Among all vertebrates different organ systems enlarge at somewhat different rates in proportion to the whole body. This is especially important in ruminants since the internal organs must assume different positions as the rumen develops. Furthermore, the very development of the rumen depends in part upon the roughage of the diet. Therefore, the calf is especially subject to its diet. Schoening (226) has provided good diagrams of the gastrointestinal tracts of different species.

Few studies have been made to establish the interrelationship between the diet and the internal organ development of the calf. Lagerlof (139) has reviewed this subject and given a series of drawings of the calf from birth until nine months of age. Transverse sections were used in the study of this organ development. Sections were made of a newly born calf, calves 1, 2, 3, 4, 5, 7 and 9 months of age as well as a full grown cow.

Lagerlof notes that the rumen occupies an unimportant position in the anterior, upper left part of the abdominal cavity at birth. At two months of age it has increased in size and sunk down within the cavity. At three months its size is proportional to that of the adult. This development assumes the calf has had hay at an early age and has not been kept upon milk and concentrates too long. Lagerlof notes that foods occupying much space and containing much water lead to the development of a large rumen with a large and pendulous abdomen. He also notes that lengthy periods of sickness with diminished feeding result in a rumen greatly diminished in size.

The reticulum develops very rapidly after birth according to Lagerlof. Likewise, the omasum is very small at birth and develops rapidly. As the liver shrinks in size relatively during the first year of life, space is made available for the omasum. As the rumen develops it pushes both the omasum and abomasum to the right and dorsally.

At birth the abomasum occupies a considerable volume of the abdominal cavity. It extends back from the diaphragm and rests on the floor of the abdominal cavity. At birth it reaches back to a plane through the seventh vertebrae. By a month of age it has undergone a relative diminution and extends only to the third vertebrae. In the adult it continues to lie on the abdominal floor.

These anatomical changes are of interest in understanding the nutrition of the calf because they are modified by the type of diet fed at different ages and possibly the degree of development of these organs modifies the utilization of nutrients.

DIGESTION IN THE NORMAL CALF

Since early times digestion in the mature ruminant has been studied but the calf has been neglected. Since every normal calf eventually develops the gastrointenstinal system of the mature animal, a brief summary of the physiology of this system may be worth while. Schalk and Amadon (225) have presented an excellent summary in this field. They have found that both grains and forages are very incompletely masticated during the eating period. They have added much to our knowledge of rumination. They found relatively few uncrushed grains involved in rumination. Water proved to be very important in the ruminant stomach. They found water tended to enter the rumen when it was drunk and did not follow the esophageal groove into the omasum. Rumination ceased in the absence of sufficient water. The ruminoreticular ingesta passes into the omasum only when it is reduced to a fine state. In the abomasum it exists as a semiliquid.

Schalk and Amadon gave some attention to calves. They found the esophageal groove seldom functioned in mature animals except under special conditions. In a two-year-old heifer this groove served to conduct milk directly to the abomasum but did not do so with water. They prepared gastric fistulae in three calves and were able to feel the groove as it functioned in one of these. They observed that it acted as a tube to conduct milk directly to the true stomach if the milk were drunk slowly but that if the milk were consumed rapidly from a pail it tended to pass into the rumen.

Wester (267) found a number of factors such as salt, sodium bicarbonate, tannin, milk and blood serum stimulated the esophageal reflex and suggested this method as a means of introducing drugs directly into the abomasum. Ross (212), however, on the basis of studies with sheep, challenged many of Wester's findings.

Trautmann and Schmidt (252) studied kids and calves. They found that even after the first week of life, cold milk might pass into the rumen. Wester had found this reflex might persist after the nursing period, if properly stimulated. Wester found the reflex for water in the case of calves was lost a few weeks after birth but Trautmann found sheep and goats retained it for several months. In general cold water tended to go directly into the rumen. After long thirst water tended to proceed directly into the true stomach. When much milk was drunk by calves, the true stomach was usually filled and after this the rumen.

Wise *et al.* (274) found milk fed to calves from pails might enter the rumen to a limited extent but if fed from nipples it never did so. They also found that after the calf was a few weeks old, water no longer stimulated the esophageal reflex. All evidence indicates that the method of feeding calves may be of considerable importance.

Few studies have been made of gastric secretion in calves. Belgowski (23) studied the gastric juice in mature cows. He found this juice secreted at a continuous rate. The secretion was stimulated somewhat for 5 to 6 hours after food was eaten. Even in fasting the secretion continued. The type of food seemed to have little influence on this secretion. The hydrochloric acid usually ran from 0.13 to 0.36 per cent. The highest value found was 0.46 per cent. He found the protein digesting power of the juice remained about the same whatever the source of protein fed.

Espe and Cannon (72) studied the gastric secretion in three calves by means of Pavlov's stomach. They found little psychic effect upon the secretion of gastric juice in spite of the preference of calves for certain foods. Shoptow *et al.* (234) did find a slight effect of feeding upon the amount of gastric juice secreted. This increased to a maximum during the fourth to fifth hour.

The idea that foods undergo no change while passing down the esophagus may have to be revised in the light of the recent sham feeding trials of Wise *et al.* (275). In calves they found milk stimulated a flow of saliva and when the mixture was collected from an esophageal fistula the viscosity was increased and the pH was lower than for either the milk (pH 6.6) or the saliva (pH 8.1). Sham fed milks contained more bacteria and were more stable since the fat rose more slowly.

Almost no attention has been given to absorption of nutrients from the tract of the calf. Little is known concerning the adult ruminant. Trautmann (252) presented an extensive review of this field. He found water and water soluble substances to be absorbed from the rumen. He stressed Italian work indicating that all glucose was absorbed from the rumen and considerable water was absorbed just before the passage of food into the true stomach. This entire field needs study in the interests of better calf feeding.

Likewise, no attention in the past has been given to the rate of development of the bacteria and infusoria in the rumen of the calf. Both Schwarz (228) and Krebs (136, 137) have reviewed the literature showing the important part played by infusoria and bacteria in modifying the protein of ruminants. Schwarz estimated that about 10 per cent of the nitrogen of the rumen was in the form of bacteria and 20 per cent as infusoria. Today we recognize the great importance of these lower forms in the synthesis of many of the water soluble vitamins as well as in the resynthesis of proteins. This whole field must be of great importance in the nutrition of the calf because of the essential nature of both amino acids and water soluble vitamins for growth. With some understanding of this field we can hope greatly to improve the wellbeing of the calf.

On the one extreme we have the beneficial effect of microorganisms in their creative activity in the rumen. On the other we have the harmful effect of bacteria in causing "scours" from their activity in the intestinal tract. In both cases the healthy calf is vitally concerned.

BLOOD COMPOSITION OF THE NORMAL CALF

Anderson *et al.* (3) compared the blood of normal calves under a month of age with those one to five months of age. They found the hemoglobin and sugar about 15 per cent higher in the younger group. From their data the following values would represent the blood of the normal calf: hemoglobin 87 per cent, non-protein nitrogen 28, urea N 12, uric acid 2, creatine 4, creatinine 1.4, sugar 88, Cl as NaCl 499, inorganic P 4.5, total acid sol. P 7.8. Ca 12.4 and plasma bicarbonate CO_2, 63 per cent. All but the last value and the first are in mg. per cent.

Many determinations have been made upon serum calcium, plasma inorganic phosphorus and serum magnesium of calves. For South African calves (Bisschop 28) the bone meal fed animals had values of about 6 mg. per cent while the controls without this supplement were considered borderline with values from 4.3 to 5.5 mg. per cent. Values as high as 9 to 9.6 mg. per cent were reported by Rupel *et al.* (216) for calves fed cod-liver oil. Wise *et al.* (276) found values of 5 to 9 mg. per cent during the first ten days after birth. These values indicate the range of the normals.

Serum Ca seems to be 11 to 13 mg. per cent for the normal calf from the data of Duncan and Huffman (54).

Duncan *et al.* (58) found the level of magnesium in the plasm varied slightly with the season. During the month of July the values were 2.26 mg. per cent ± 0.010, while in November they were highest with values of 2.55 mg. per cent ± 0.017. In one report Duncan *et al.* (57) reported figures as high as 4 mg. per cent for a calf kept in the sunshine. The studies of Knoop *et al.* (131) also indicate that magnesium values above 2 mg. per cent are normal. For calves Groenewald (89) found values quite constant at 3 mg. per cent. Methods for Mg are not entirely satisfactory and may explain these differences.

According to Cunningham (42) the tissues of both calves and adults contain 0.06 to 0.12 per cent of the dry matter in the form of Mg.

Groenewald (89) studied the blood of 15 calves, 12 of which were heifers, to determine normal changes during the early weeks. Calcium fell within the range of 11–13 mg. per cent during the first 6–7 weeks and then dropped to 9–10 mg. per cent. P ran about 7 for the first 7 weeks and then rose to about 8 mg. per cent. Potassium started at over 125 mg. per cent the first week and dropped to about 50 in the 12th week. Sodium started at 230 the first week and increased to 320 mg. per cent in the 12th. Chlorine was steady at about 310 mg. per cent.

Kennedy *et al.* (129) studied the composition of blood in relation to gestation, lactation and age. For the blood of heifers they found mean values of the following: non-protein nitrogen 32–38 mg. per cent, glucose 52, Ca 10.9, and P 5.4. Blood sugar values in calves ran about twice as high as in cows. Before feeding the value might drop to 81 while an hour after feeding this might rise to 129 mg. per cent. By glucose feeding to very young calves this level could be raised to 150–200 mg. per cent. As calves grew older the response was less until at the age of 6–9 months the rise is from 60 to a peak of 90 with a return to normal in about 3 hours. The blood sugar of the normal cow runs 40–60 mg. per cent. Dukes (53), Turk and Work (254) found 51 mg. per 100 cc. of whole blood of apparent glucose but only 28 mg. of true glucose in cows.

Bechdel *et al.* (15) have furnished tables that show the variations in the Ca and inorganic P in the serum of calves when blood samples were taken at regular intervals for about 5 months. Although these experiments were made in connection with the development of rickets, they are useful in establishing variability.

Duncan *et al.* (57) determined the magnesium in the blood of normal calves from birth until a year and a half of age. They made more than 2,000 determinations and established a mean of 2.4 with variation from 1.6 to 3.8 mg. per 100 cc. of plasma.

THE COMPOSITION OF THE ENTIRE CALF BODY

Few attempts have been made to analyze the entire bodies of calves due to the trouble of grinding and selecting representative samples. Haigh *et al.* (98) made such determinations. Their tables of the weights of the various parts of Jersey and Hereford calves are too extensive for reproduction but very useful for reference. Their tables of the moisture, fat, N, P, and ash in the whole body and its parts are also very useful. From a dam on a medium plane of nutrition the calves were composed of moisture 73.3 per cent, fat 3.2, nitrogen 2.8, ash 4.6 and phosphorus 0.79. In their studies the plane of nutrition of the cow modified the distribution of the fat in the calf at birth. This might be of some importance. Thus, the bones of the calves from the dams at the higher planes contained 4.7 per cent fat while at the lower plane this value was only half of this or 2.3 per cent. The livers of the "higher plane" calves ranged in weight from 666 to 902 grams while on the "low plane" the range was 240 to 646 grams. The distribution of fat in the body of an animal may be of much importance during the early days of life. The fact that the diet of the mother can modify the storage of fat in the bones of the calf is of interest.

The distribution of copper in the body of the calf at birth has been studied by Rusoff (217). According to his results the calf body contains about a fourth of a gram of copper. Most of this element is found in the flesh, bones and liver. Four calves were used. Some of the values found, such as those for the blood, suggest considerable errors either in obtaining the samples or in analytical procedures. Rupel *et al.* (214) state that the normal hemoglobin for a calf is 9 to 13 grams. Many studies have been made of the composition of the bones of calves. Among the earliest are those of von Bibra (27). He found the inorganic material increased as the calf matured. He found some F even in the bones of the foetus. In recent studies Evans *et al.* (73) have found that the F is relatively low in calf bones. Thus, veal calves have 25–51 ppm. of F in their bones while the mature cow has 177–202 ppm. of F in their bones. Bone meal in turn is about twice as high as this value for the bones of the cows. These workers found F even in the embryo.

Kruger and Bechdel (138) killed normal calves at ages of 60, 90, 120, 150 and 180 days for bone analyses. These calves ranged in weight from 135 to 449 pounds. They studied sampling by analyzing a complete humerus on one side and a section from the bone on the other. They found sections rather unreliable. Typical examples for two different bones, from their data are the following:

Age at death	*Water* *Per cent*	*Fat* *Per cent*	*Ash* *Per cent*	*Bone used*
60 days	55	5	20	Femur
	48	2.6	29	Rib
180 days	39	19	25	Femur
	41	3.4	32	Rib

The authors made some studies of the frontal bones but concluded these calcified too early to be useful in studying bone changes. This accords with the well known fact that the head is nearer maturity at the time of birth than other parts of the animal body. These data also indicate that the long bones are the more important in the storage of lipids.

Some workers who have studied bones extensively believe that the ribs are the most satisfactory for the study of changes in ash content that characterize calcification.

Moore (182) has provided some data upon the normal level of carotene in the blood of healthy calves. This level seems to lie between 0.2 and 0.7 γ although on pasture he found values as high as 15 γ per cc. of plasma.

Few other normal data are available at present to evaluate the effects of diet upon the calf. Delaune (50) made a limited study of the erythrocytes and leucocytes and gives 8.7 millions and 10.7 thousands respectively as the normal. In his differential count he found lymphocytes 64 per cent, monocytes 12 per cent, neutrophiles 19.6 per cent and eosinophiles 3.3 per cent.

PROTEIN REQUIREMENTS

The calf since it is a ruminant represents an animal unique from the common omnivora such as the dog and rat which have been extensively studied in nutrition laboratories. For the first few weeks of life the calf resembles the dog very much in the manner it digests its food. After a few weeks, however, it transforms its gastrointestinal tract to the remarkable structure found in the adult.

In the course of this transformation many species of infusoria and bacteria are gradually established in the rumen as this interesting stomach develops. With the establishment of this symbiotic relationship with lower organisms the calf attains the power of providing its body not only with water soluble vitamins but also with proteins. These proteins may represent the activity of microorganisms that break down and reconstitute the proteins of feedstuffs or the proteins found in the bodies of the microorganisms by new ones created from simple nitrogenous compounds found in plants such as the amides or fed in the diet in the form of urea.

Today very little information is available concerning the rate at which the bacteria and infusoria establish themselves in the rumen. Furthermore, little is known about the potential synthetic powers of the different species that do establish themselves. These forms that are established may be partly determined by the nature of the diet and in turn they themselves may partly determine the nature of the material in the rumen to be digested. This will affect the wellbeing of the calf since the supply of essential amino acids and water soluble vitamins may depend upon the mircoorganisms that are able to thrive.

Since the protein requirement of the calf can be supplied in part by simple compounds such as ammonium salts and urea, the term "nitrogen requirement" may be more appropriate. For more than half a century the question of the utilization of nitrogen compounds has been debated. Much of the experimental work has been seriously confused because protein rich foods often carried vitamin supplements while simple nitrogenous compounds did not. Thus, in Rudzki's studies (213) with rabbits in which he claimed he could maintain them for 6 to 7 weeks on a mixture of uric acid, starch and fat, much of this maintenance would depend upon the body reserves of fat soluble vitamins. From early times the German workers assumed the bacteria and infusoria of the rumen could synthesize protein from simple compounds of nitrogen. The chief question that arose was whether or not the protein thus made could be digested after it passed from the rumen into the true stomach. Max Muller (187) found the dog could be kept in nitrogen balance by proteins synthesized *in vitro* by organisms taken for the rumen. Thus, he concluded these proteins were well utilized although from time to time the Germans continued to suggest that there were anti enzymes in the bacteria and infusoria that prevented their digestion by pepsin in the stomach. No good proof for this point of view was ever presented except that herbivora could not be kept upon diets with all the protein replaced by simple compounds.

During the first World War the German interest in feeding such compounds as urea to cows and sheep increased. This led to many studies. Schwarz (228) reviewed the literature of the past hundred years showing the growth of our ideas concerning the part lower forms play in rumen synthesis. Schwarz attempted to measure the nitrogen present in the rumen in the form of bacteria and infusoria. He first filtered through paper some of the fine suspension of rumen contents. He assumed the infusoria and some suspended protein were retained on the paper while soluble protein and nitrogen compounds as well as bacteria passed through. He then determined infusoria nitrogen by artificial digestion of the retained compounds with pepsin and HCL. He assumed this pepsin digestion would only attack infusoria protein. Thus he could measure it. To determine bacterial protein he passed the filtrate through a Berkfeld filter and assumed that the material retained was bacterial.

On the basis of these somewhat questionable fractionations he concluded about 10 to 12 per cent of the rumen nitrogen was in the form of bacteria and about 20 per cent in the cells of infusoria. Thus, he estimated about 256 grams of protein would be present in these living organisms in each 100 kg. of rumen contents.

Fifty years ago Hagemann (97) summarized the part lower forms play in the protein cycle as follows: "Organisms live very well in food solutions without protein, like those with amides: they live therein and build amides into body protein. Later this is digested so that asparagin from the feed becomes protein through the life processes of the micro-organisms. If amides are lacking the micro-organisms lack nitrogen and attack the true protein, thus taking them from nutritional use." The last statement would be questioned in the light of modern research.

Krebs (136, 137) has presented a good review of this whole field of the utilization of nitrogen compounds by animals. He indicates that many factors control the lives of the infusoria and bacteria. Thus, the infusoria seem to decrease as the protein of the ration increases although no one has shown whether this is due to the protein or the vitamins that accompany it in the ration. The form of sulfur in the ration may also be of considerable importance.

Sheep seem able to utilize about half protein and half N in simple compounds for growth. Krebs called attention to the need for growth experiments lasting a considerable period of time. In 1937 Fingerling *et al.* (75) presented good evidence from balances with calves covering a period of four months that these animals were able to convert urea into body tissues.

More recently the Wisconsin workers Hart *et al.* (101) have provided additional evidence that growing calves can utilize urea and ammonium salts in the formation of body proteins. They took advantage of the early German observation that diets rich in carbohydrates stimulated the utilization of nitrogen compounds. In their second experiment their basal diet consisted of yellow corn 20 per cent, ground timothy 47.5, starch 24, corn molasses 10, bone meal 2, salt 1 and cod liver oil 0.5 per cent. When this diet was fed alone the calf tended to fail rapidly but when supplemented with 1.4, 2.8, or 4.3 per cent of its weight of urea the diet produced good growth until the calves were a year old. The basal diet also provided good growth if supplemented with 11 per cent of its weight of ammonium carbonate or casein.

In vitro studies of the type employed by Muller (187), Wegner *et al.* (262) indicated that most of the urea was utilized by the organisms from the rumen in about 25 hours. They found the ration of the cow a rather poor medium. They found little influence of the protein level in contrast to early German work. All carbohydrates proved equally good except cellulose.

Much additional work needs to be done to establish the optimum conditions for developing and maintaining the best flora in the rumen of the growing calf in order to get the maximum value from either plant proteins or nitrogen compounds.

Furthermore, Mazincesco (156) has presented evidence that the minimum protein requirements of swine can be lowered by feeding such compounds as glycine, aspartic acid, alanine, glutamic acid, asparagine, acetamide and succinamide as well as cystine. This indicates that there may be some utilization of these compounds in the animal body that is independent of the action of micro-organisms.

In Armsby's book (6, table 80) is an excellent summary of data showing the rate at which young animals are able to add to their body protein. During the first couple of weeks the young calf can add about ± 2 per cent per day while at the age of two months this rate has declined to less than one per cent. At the end of a year it is about one-fifth of one per cent.

For a hundred years men have attempted to formulate feeding standards, usually based upon meager data, the limited experience of the usual professor in rearing animals, and considerable arithmetic. One feels inclined to follow McCandlish (161) after he has made a critical survey of the protein literature, and quote Armsby (6, p. 691), "But the human mind craves a receipe and there has been a persistent tendency to substitute for the study of the principles of nutrition a series of exercises in applied arithmetic."

In the past there were numerous chances for error in evaluating the protein needs of the growing animal. In the first place there is the ancient debate of whether feedstuffs as sources of protein should be rated on the basis of their nitrogen content or of their true protein. Today the pendulum is swinging toward "total nitrogen." In early experiments efforts were made to allow each calf an equivalent of nutrients for energy but little attention was paid to providing equal allowances of water soluble and fat soluble vitamins, inorganic nutrients and essential amino acids. Today we appreciate that the bacterial growth in the rumen may be profoundly modified because these bacteria have specific requirements for nutrients. Even today we have little evidence that would help us design experiments with equivalent nutrients to secure optimum growth of the rumen flora. Much fundamental work must be done before we can establish data to guide us in keeping the protein the only variable because we must define the nutritional needs of the micro-organisms that live in the rumen.

In general the requirements of calves for protein during growth have been set at high levels. A typical example can be found in the table of Kellner (127). Fingerling (76, 77) thought the prevailing standards were too high. After a number of years of experimenting he concluded 1.5 kg. of protein per day per 1000 kg. of live weight was adequate if the ration had a starch value of 12–13 kg. per day per 1000 kg. of live weight.

Fingerling extended the early observation of Soxhlet and came to the same conclusion that the young calf is able to convert into body tissue 80–90 per cent of the proteins of milk during the first couple of weeks of life. Fingerling was able to keep this conversion high for a longer period of time if he fed a lower level of protein but kept the energy intake adequate so that the calf did not have to consume protein to supply its need for calories.

The laudable attempts of the National Research Council to organize co-operative experiments in determining the protein requirements of calves were reported by Armsby (7) and Forbes (81). Due to the small number of experimental animals used, little progress was made. The data, however, did indicate that some calves could make good growth upon relatively low protein diets. Future investigations will be needed to determine whether this was due to hygiene, unappreciated vitamin supplements, or other unrecognized variables. Animals from 6 to 16 months of age gained 0.7 to 1.5 pounds per day with protein intakes of 1.2 to 1.7 pounds of digestible crude protein per 1000 pounds of live weight. This accords with the findings of Fingerling and shows that protein economies can be affected if the basic information is established and feed costs demand it.

The eight years of research at Missouri reported by Swett *et al.* (245) lend support to the use of higher protein levels in rearing calves, especially Jerseys. If 13.4 or 15.8 per cent of the net energy of the diet were in the form of protein the Holsteins could exceed the normal growth rate. Even with this figure at 10.4 per cent the growth rate was 94 per cent of the Missouri normal. In the case of the Jerseys, however, the normal was barely reached at the 18 per cent protein level and at the 12.9 per cent level the growth rate was only 80 per cent of the normal.

These authors attempted to determine if they could maintain a normal growth rate by progressively decreasing the protein level as the calves grew older. The results were irregular but in general favored the higher protein levels. As a rule energy levels well above the requirements favored growth at a constant protein level. The data from the Holsteins compares favorably with that for rats fed a high quality protein but the Jerseys seem to have a higher protein requirement than other species that have been studied such as rats, mice, fish and dogs.

In general, long-time growth studies are probably the most satisfactory for determining the requirement of calves for protein. As knowledge advances it may prove possible to employ synthetic diets in rearing calves in order that there may be no confusion between the effect of proteins and other constituents that may accompany them in natural feedstuffs.

Other techniques that are worthy of wider use are both the chemical balance determinations and the analysis of the entire carcass. Both of these have been used to a limited extent.

The chemical balance method has been severely criticized because short-time studies have been made and conclusions drawn from them that are invalidated because the period was too short and the individual too variable. However, the balance method is a useful tool to use in conjunction with long-time growth studies. In a short period of time it tells the extent of the digestion and absorption of protein mixtures. This is well illustrated by some recent studies at Cornell with another species, namely swine. These swine were being fed large quantities of raw eggs that had failed to hatch in the incubators. Since it is well known that rats, dogs and men utilize only about half of the protein of raw eggs, the possibility existed that half of the protein of these eggs was being wasted. By means of balance studies it was established within a month that growing swine utilize the protein of raw and cooked eggs equally well. Such evidence was conclusive and could be obtained in the course of growth studies without waiting for a large fraction of the growth period to be completed.

Some interesting results have been found in nitrogen balance studies with calves. Carr *et al.* (38) ran three-day balances using five different combinations of plant and animal proteins. They found the retention of nitrogen to range from 23 to 41 per cent. The protein of milk seemed to be best and that of dried blood the poorest from the point of view of utilization. The great variability of results suggests that the processing of proteins deserves great study in order to provide them in calf diets so that they are in condition for the optimum utilization.

Dried blood affords an excellent example of the need for careful attention to processing. Caldwell (33) found that fresh blood was much better utilized by calves than dried blood. When his calf was used for a balance trial with 40 per cent of the N from fresh blood at the age of 100–110 days the nitrogen was distributed as follows: feces 31.4, urine 41.2, and retained 27.4. In the next 10 days with 33 per cent of the N from dried blood the distribution was feces 46.2, urine 22.6, and retained 31.1 per cent. His conclusions were probably drawn from the higher fecal nitrogen. He concluded a daily ingestion of 59 grams of N gave about the same results as 93 but that 48 grams was too low. These studies were made with calves under 5 months of age.

In a later study Spitzer and Carr (241) found that much of the dried blood was lost in the feces of the calf. Nevertheless, they also got a higher per cent of nitrogen retained upon a dried blood than upon a dried milk diet. Winter (273) has presented an excellent review of the literature covering the nutritive value of dried blood. From experiments with rats and swine he concluded the digestibility was decreased by increasing the temperature in the processing.

These data indicate the possibility of improving the utilization of the proteins fed to calves not only by combinations to provide adequate supplements among amino acids but also by improvements in drying or other processing. In the case of soybean meal the heat treatment is probably advantageous, while in the case of meat and blood products, the body is unable to utilize efficiently proteins that have been subjected to high temperature drying.

The analysis of the entire carcass of large animals such as calves has always been such a task that this method has been little used. The extensive development of bone-grinding machinery in recent years for use on fox ranches has provided inexpensive methods for reducing bones for analytical samples. Any journal for the fur-bearing industries carries advertisements for such machines and they should make it possible to analyze the whole bodies of animals of large size in order to measure the storage of nutrients from experimental diets.

THE UTILIZATION OF CARBOHYDRATES

The aspects of carbohydrate metabolism that have been studied in the case of the calf are the following: (a) the utilization of cellulose, (b) the digestion and absorption of starch and (c) the utilization of simpler sugars such as glucose and lactose.

The basic studies made sixty years ago concerning the utilization of cellulose by the adult ruminant paid no attention to the calf. Today we know nothing about the development of conditions that permit the optimum use of cellulose at an early age. Furthermore, we have neglected in all fields the processing of cellulose so that herbivora can get more energy from it. Karrer and Schubert (126) found that the breaking down of cellulose by the cellulase of the snail, might vary from 7 to 70 per cent, depending upon the source of the cellulose and the chemical processes to which it had been subjected.

Many attempts have been made to rear calves without roughage. Most of these will be discussed later, under the subject of milk feeding. In 1893 Sanborn (220) reported his attempts to rear calves without hay. He was able to get moderate growth in calves for a short time. He maintained a steer for about eight months on grain alone. He found the steer ceased rumination on such a ration. Davenport (46) reported studies in which a calf was kept for 6 months on milk and grain. At the age of 6 months the calf was eating a half bushel of grain daily but still it failed to thrive. His calves also failed upon milk alone. Today we realize these deficiencies were multiple ones and not due to the lack of roughage alone.

Mead *et al.* (178, 177) have attempted to rear calves without roughage. In heifers fed for 18 months without roughage they found digestion normal. The addition of paper pulp to the diet did nothing but improve the digestion of the fiber added. These calves were reared and kept for six years without roughage in the form of hay.

Some years ago the use of regenerated cellulose (McCay, 168) was introduced in order to make it possible to study the roughage needs of herbivora without having the source of roughage provide other nutrients. Synthetic diets were developed for herbivora by McCay and Woodward (170), McCay, Ku *et al.* (169), Madsen *et al.* (149). Herbivorous animals have been reared for long periods upon such diets but they have never proved as satisfactory as those composed of natural feeds. This indicates that there are still deficiencies in the feeding of herbivorous species that are unrecognized today.

Johnson (121) fed calves synthetic diets containing 5 to 20 per cent regenerated cellulose between the ages of 13 and 130 days of age. Fair growth was obtained but the calves were rough in appearance and not normal. In part this abnormality was probably due to the inadequate level of magnesium in the diet but in part it was unexplained.

The digestion of starch by the calf has interested workers for nearly a century but little is known about it today. In the early substitute for whole milk that Kellner (127) credits to Liebig, wheat flour was treated with ground malt supposedly to help the calf digest the starch. Kellner in the same work describes the use of potato starch treated with malt, as a food for the calf. According to Hittcher (108) the French had long tried to replace the butterfat of whole milk by starch in feeding calves. Hittcher ran an experiment with 37 calves to determine if there was any advantage in treating starch with malt when this starch was fed as a supplement to skimmed milk. His experiments lasted for 15 weeks and very young calves were used. The mean age was 7 days at the start of the experiment. Fifteen of his calves died from various causes during the period. The calves fed the starch treated with diastase did slightly better for the first four weeks. Due to the variability it is doubtful if the data of Hittcher yielded significant differences. The individual responses may have been the result of reactions to such factors as vitamin A deficiency rather than to the malting of the starch. The survival of any of the calves upon a diet so poor in vitamin A is surprising.

Hanne (100) reported good results from feeding skimmilk and potato starch treated with malt diastase after the starch had been boiled. Fingerling (78), however, had much trouble with intestinal disorders. He believed this was due to injury to the linings of the stomach and intestines. It is surprising that any of these calves lived on account of the low level of vitamin A in the diet.

Eddin (66, 67) reared calves upon skimmed milk supplemented with starch treated with diastase. His innovation consisted in preserving the mixture by adding formalin in a proportion of 1:10,000. Possibly formaldehyde should be given more study as an agent in animal feeding to check unfavorable bacterial action before the food is ingested. The toxicity of formaldehyde is relatively low especially after it has had time to combine with protein.

Scolz (229) fed calves flaked potatoes starting at an age of 6 days. Starting with 10 grams of the potatoes, the amount was increased to 280 grams at 44 days of age. In spite of feeding some cod-liver oil the calves suffered from scours. The qualitative test for iodine showed little starch in the feces at any time although the feces from the scouring calves were very high in fat.

Lindsey and Archibald (144) got fair results in rearing calves upon grain mixtures to which had been added 14–19 per cent of corn starch and 5 per cent of corn sugar. Shaw *et al.* (230) made an attempt to determine the amount of starch digested by calves at different ages. When calves were fed 80 grams per day of corn starch, presumably raw, they found only 20 to 22 per cent of it digested when the calves were 4 to 7 days of age, but when the calves reached the age of 3 weeks, 90 per cent of it disappeared from the gastrointestinal tract. These experiments need much extension to make certain the starch was not lost through fermentation. Furthermore, calves may be like dogs at an early age in suffering disturbances in the intestinal tract from the ingestion of large amounts of raw starch.

Little is known about the utilization of sugars by calves because most of the studies are complicated like the starch feeding trials by deficiencies in vitamin A. A number of studies have claimed good results from feeding whole milk supplemented with corn sugar (Martens, 152). As one might expect the results of supplementing skimmilk with glucose are conflicting (Golf and Schwabe, 86).

Little attention has been paid to other sugars such as lactose and sucrose. Lindsey and Archibald (144) claimed lactose at a 30 per cent level gave favorable results but was too expensive to be of practical importance. Robinson *et al.* (211) found that lactose favored the storage of calcium when this carbohydrate was fed with bone meal to calves. This interesting observation should be extended.

Bunger (32) found buttermilk supplemented with lactose or glucose gave better results than skimmilk.

Finally the studies of Usuelli (257) indicate that considerable amounts of starch are converted in the rumen into glycogen. The part this plays in the utilization of starch by the growing calf deserves attention.

FAT UTILIZATION BY CALVES

Few studies of the part fat plays in the diet of the calf have been made. From early times attempts were made to substitute products rich in fat for the fat of milk. Thus, flaxseed meal was a favorite produce although today it is hard to understand how the calves survived with so little vitamin A until they began to eat hay. Well before Frankland (82) established the high calorific value of fat, Crusius (41) found that the food value of milk was doubled if the fat were left in. His experiments were done with calves.

Fingerling (78) supplemented skimmed milk with peanut oil, peanut meal and flaxseed meal. All of these supplements proved satisfactory for calves. Various attempts have been made to feed skimmilk supplemented with oils such as cod-liver oil or tallow. Zorn and Richter (280) reported such attempts. In the first trial cod-liver oil threw the calf off its feed quickly. Neither the tallow nor lecithin oil emulsion proved as good as butterfat. Even combinations of cod-liver oil and tallow were not entirely satisfactory.

The literature in this field has been reviewed recently by Gullickson *et al.* (94). These workers fed grade calves upon skimmilk in which various fats were emulsified at a level of 3.5 pounds of fat per 96.5 of milk. In one trial calves were allowed only a low fat diet consisting of "100 pounds of ground molasses beet pulp, 50 pounds of dry skimmilk and 25 pounds of cerelose." No hay was allowed but the equivalent of 25–35 cc. of cod-liver oil was fed daily.

Calves fed animal fats as lard, butterfat or tallow did better than those given soybean oil, cottonseed oil or corn oil. Calves fed butterfat responded the best. Gains of only 0.31 to 0.40 pounds daily were made by those given plant oils.

The whole field of the digestion and absorption of fat by calves has been little studied. Some evidence indicates that there may even be a little fat absorbed before the fat enters the true stomach. Norris (192) found the excretion of fatty acids in the feces of the calf was 3 to 4 times as high when gruel was fed compared to whole milk. This indicates the feed of the calf may make extensive changes in the chemical reactions taking place in the intestinal tract. Haecker (95) tried rearing calves upon an emulsion of corn oil in skimmed milk. If the fat was used at a level of 3 per cent it proved too laxative but at 2 per cent it was quite satisfactory.

Nothing is known about the amount of fat that can be digested and absorbed by a calf. A growing lamb or rabbit can be fed as much as twenty per cent by weight of its diet in the form of fat and still make good use of it. Possibly the calf and cow can also tolerate higher levels than have been fed.

Calves seem much more resistant to the injurious effects of cod-liver oil than other herbivorous species such as rabbits, guinea pigs, goats and sheep. Madsen *et al.* (149) and Turner *et al.* (256) found calves fed a poor grade of timothy and a grain mixture grew normally if fed a supplement of 0.7 cc. of cod-liver oil per kg. live weight per day but died if this level was increased to 2 cc. Cod-liver oil was fed by Davis and Maynard (47) to dairy calves from birth to 6 or 9 months of age at levels up to 0.7 grams per kg. live weight without any injury except some minor changes in the muscles of those fed the higher levels. In most herbivora as well as in milking cows the injury seems to result from feeding levels above a third of a cc. per kg. of live weight per day.

By the use of the electrocardiograph Agduhr *et al.* (1) claimed they found evidence of injury to the hearts of calves from feeding cod-liver oil. Barnes *et al.* (11) found no evidence for such injury but did find changes in the electrocardiograms as the calves grew older. Various factors may modify the electrocardiogram in the calf. Sykes and Alfredson (246) found both age changes and also some due to the diet low in vitamin K.

Inasmuch as a number of studies have now indicated that the muscle degeneration found in herbivora after feeding cod-liver oil is in part due to destruction of vitamin E within the animal's body, these cod-liver oil data produce some evidence that the calf has a lower requirement for this vitamin as well as more resistance to cod-liver oil injury than sheep and goats. Possibly cod-liver oil is rapidly digested and absorbed by the calf before it has a chance to exert its destructive effect upon the vitamin E of the diet.

FAT SOLUBLE VITAMINS

Nothing is known about the requirements or metabolism of fat soluble vitamins E and K but some attention has been given by researchers to vitamins A and D.

Vitamin A. In spite of the fact that the calf is born with a very poor reserve of vitamin A, it seems able to survive upon low levels of this vitamin until it can eat hay, if it is able to start life by consuming the colostrum, and building an initial reserve of this factor. Although experiments from several laboratories indicated clearly as early as the summer of 1913 that vitamin A deficiency would often destroy the eye before the animal died, this subject was given little attention until about 10 years later by those concerned with feeding calves.

In 1924 both Hart *et al.* (102) at Wisconsin and Eckles (62) at Minnesota noted that calves might be born dead and blind in cases where the cow had been maintained upon a diet deficient in vitamin A.

The excellent paper of Jones, Eckles and Palmer (123) showed the importance of vitamin A in the nutrition of the calf. To provide an A-free diet these workers treated whole milk with hydrogen peroxide and then bubbled oxygen through it. Skimmed milk was freed by bleaching in the sunshine. In summer the calves were left in the sunshine and in the winter D was furnished by cod-liver oil freed from vitamin A. If their diet contained 40 per cent wheat straw, the calf could grow normally and assays with rats showed that 35 per cent of this straw provided the rat with adequate vitamin

A. The time for their calves to break down ranged from 2 to 6 months. The many symptoms that accompanied this decline included failure to grow, pneumonia, nephritis, sclerosis of the liver, and necrosis of the rumen as well as blindness. Twenty cc. of cod-liver oil, or if it was present as about 0.8 per cent of the dry feed, allowed adequate vitamin A. Roughly, then, the calf would need less than 16,000 units per day of vitamin A.

In 1928 the edema that accompanies "A" deficiency was studied further by Bechdel *et al.* (19) while Reed *et al.* (209) found the blindness that developed in heifers fed cottonseed meal could be prevented by feeding more hay, thus increasing the vitamin A of the diet. Bechdel (16) found this blindness could also be prevented by feeding 25 cc. of cod-liver oil daily.

Schultz (227) compared the effect of A-deficient diets upon horses, rats, children, cattle and swine showing the common origin of eye symptoms. Mead and Regan (178) in rearing calves without hay ran into A deficiencies after about 7 months even though some whole milk was fed until the calves were 6 months old. In 2 to 7 days their deficient calves responded to cod-liver oil feeding and sight was recovered in 1 to 3 weeks.

Moore and Hallman (181) observed the white spotted kidneys of calves fed diets deficient in vitamin A and noted they resembled those found in calves that are deprived of colostrum. Furthermore, Moore (182) by means of the ophthalmoscope observed a swelling or edema of the nerve head leading into eye in calves fed a low intake of carotene. As an A-deficient diet he fed skimmed milk and a grain mixture of barley 240 lbs., rolled oats 180 lbs., wheat bran 180 lbs., linseed oil meal 60 lbs., and salt 8 lbs. Calves put on this ration when 40 to 90 days of age showed signs of night blindness in 48 to 73 days. This could be cured in 5 to 18 days. The plasma level of A might drop as low as 0.13 γ. This increased to 0.2 γ from feeding 12 to 13 γ of carotene per pound of body weight.

Calves which were fed 28 γ were very thrifty and their plasma level was about 0.7 γ. On pasture, Holstein calves showed plasma levels as high as 15 γ per ml. of plasma.

Guilbert and Hart (91) estimate the needs of cattle at about 29 γ per kg. of live weight. They found that the injection of carotene dissolved in olive oil subcutaneously, led to relief of eye symptoms in a deficient calf, but in general recovery was very low. Other workers have given a slightly lower requirement of 11 γ per pound of live weight (Flora *et al.* 79, Ward *et al.* 259).

The question is frequently raised concerning the need of calves for supplements of vitamin A beyond that furnished by normal feedstuffs. Early experience with diets very low in A indicates the calf can bridge the period between colostrum feeding and the consumption of hay with quite modest allowances of vitamin A. Also Dahlberg and Maynard (44) could find no evidence that the addition of a concentrate from cod-liver oil to furnish more vitamins A and D was advantageous. Insko and Rupel (119) found that the addition of 2 per cent of cod-liver oil to a good ration was of no advantage in rearing calves to 6 months of age.

A possible relationship between vitamins A and C is suggested by King *et al.* (130) in a recent report from Wisconsin. A few years ago Guthrie at Cornell found that a cow on a diet rich in cod-liver oil increased the apparent vitamin C of its milk very much but he could never repeat this result with some other cows. King *et al.* have found that calves fed diets deficient in vitamin A so that the plasma value drops to 0.18 γ also exhibit a parallel drop in both the vitamin C of the blood and a decline in the excretion of this vitamin. Furthermore, the papillary edema described by Moore (182) was prevented by feeding carotene and injecting vitamin C but not by either procedure alone. Inasmuch as the calf is known to synthesize vitamin C, these interrelationships are of considerable importance since a deficiency of A might lead to two deficiencies.

Sampson *et al.* (219) report the loss of 84 calves in a Hereford herd fed a winter ration of wheat straw and yellow corn. These calves had fatty infiltration of the livers, low inorganic P in their blood serum, low storage of vitamin A and some hypoglycemia. These losses were probably a reflection of several deficiencies not only in vitamins but in minerals and protein.

Shepherd and Converse (232) have provided good illustrations of the effect of a deficiency in vitamin A in retarding the growth of calves. They concluded 4 to 8 cc. of cod-liver oil would satisfy the calf's need for vitamin A.

Vitamin D. Rickets in calves seem very similar to this disease in other species. Marek (151) has compared rickets produced in calves, swine, dogs and other species. His work should be consulted for descriptions of the histology and pathology of the disease. His section concerning the biochemistry deals mostly with swine. The number of experimental studies with calves has been limited because the dog, the rat and the pig are easier to use for the experimental production of rickets.

Bechdel *et al.* (15) produced rickets in calves by feeding a diet based upon the one commonly used for rats. This consisted of yellow corn 64, corn gluten 24, casein 8, chalk 3 and salt 1. When the calves were killed at about 200 days of age and the ash of the femur, humerus and rib determined, it proved to be about 10 per cent higher in the normal running 55 to 59 per cent of the moisture-free, fat-free basis. A more palatable rachitogenic ration for calves consisted of yellow corn meal 54, corn gluten 24, rolled oats 10, casein 8, chalk 3 and salt 1. Beet pulp was fed as roughage. The calves were given whole milk for a month and skimmed for 3 months before they went on the diet. Upon such a diet calves seldom developed rickets if fed 25 cc. of cod-liver oil daily or if either the calves or their feed were irradiated. In this study the per cent of ash in the whole femurs of the rachitic calves was 52 to 56 while in the other calves these values ranged from 58 to 62.

These workers found the calf could get adequate D from oat straw or hay that had been irradiated. X-ray studies of the bones of rachitic calves showed poor calcification. In these calves the blood Ca dropped from 10 to 8 and in the inorganic P from 7 to 4–5. A pound daily of sun-cured alfalfa hay was not enough to cure the rickets in these calves but 2½ pounds daily was adequate. Good oat straw proved about as good as the hay for the calves.

Duncan and Huffman (54) studied the effect of ultraviolet irradiation upon the blood chemistry and mineral retention of calves. After a calf had developed rickets in early March it took more than a month of exposure to sunshine to relieve it. When a calf was getting adequate exposure to sunshine they found more than half of the Ca and P of the diet might be stored but if it were not irradiated there would be little storage. These authors decided that the analysis of a rib bone was a good method of evaluating the response to antirachitic treatments.

Bechdel *et al.* (18) determined the needs of the calf for vitamin D. A level of 135 units of D per 100 pounds of live weight from birth to 7 months of age proved insufficient, 300 units seemed adequate. Either irradiated yeast or cod-liver oil could be used to furnish this "D." In these studies the rickets were produced by feeding calves a concentrate of yellow corn 51, ground oats 20, corn gluten meal 20, linseed oil meal 4.25, soybean oil meal 4.25 and salt 0.5. Long *et al.* (148) found calves needed only 30 to 40 U.S.P. units of vitamin D per 100 pounds live weight if the minerals including Mg were fed in adequate amounts.

Krauss and Knoop (134) found calves could be irradiated for 45 minutes daily on the head or back with 82 to 84 microwatts per square meter of ultraviolet light and thus secure protection equal to that afforded by two hours of sunshine in midsummer. The blood Ca of such calves was similar to that of the normal with values ranging from 8.8 to 10.4 while the inorganic P values were also normal, running from 6.7 to 8.4.

Marek's (151) description of the pathology of rickets in calves has been amplified by the Michigan workers (Bechtel *et al.* 21). Among the many symptoms listed are lowered Ca and P of the blood, bowing forelegs, swelling of the knee and hock joints, humping back, paralysis after the fracture of vertebrae, fractured femurs, dragging of rear feet, tetany and irritability, retarded growth, anorexia toward foods but not toward milk, excess fluid in the joints and distended gall bladder. Some of these symptoms may have been related to Mg deficiencies.

Wallis (258) found that even mature cows developed deficiency symptoms upon a diet very low in D. The blood Ca and P dropped. Retention of these elements was poor and the cows tended to become stiff.

Very little "D" is stored in calf tissues (Dutcher and Guerrant 59). This conclusion was based upon rat assays made by feeding the liver and blood of calves fed cod-liver oil and irradiated yeast.

Duncan and Huffman (56) were able to kill calves by feeding very high levels of 1000 X viosterol. Under such conditions the blood Ca values rose to 10 to 13 mg. while the blood P increased to 5–11 mg.

Gullickson and Eckles (93) were able to keep calves for two years in darkness without injurious effects, showing that light furnished no essentials other than vitamin D.

Some additional discussion will be given to vitamin D in the section devoted to minerals.

THE WATER SOLUBLE VITAMINS

Vitamin C. Inasmuch as no bacteria are known that synthesize vitamin C it is unlikely that herbivora have any means of synthesizing this vitamin in the gastrointestinal tract. In 1924 McCandlish (164) tried to maintain a calf upon milk alone by supplementing this with tomatoes but the calves failed unless given alfalfa hay. In this same year Thurston *et al.* (250) found that calves remained normal upon a diet that produced scurvy in guinea pigs. Later (249) these workers reared calves from birth upon a diet so low in vitamin C that it produced scurvy in 30 days when fed to guinea pigs. After heifers were maintained for a year upon a diet low in C, their livers still contained a considerable amount of this vitamin. The work of Phillips *et al.* (201) previously noted indicates that the vitamin C synthesis is adequate where enough A is provided.

Vitamin B. In considering the requirements for water soluble vitamins, the herbivora have a unique position. During the first weeks of life the young ruminant probably must depend upon its food for these essentials and can be regarded as functioning like a young dog or rat. After the bacterial flora are established, however, the animal is in position to satisfy the requirements of its body for the fractions by means of bacterial synthesis in the rumen. The problem is still different in the horse and rabbit but cannot be discussed here. After the flora of the rumen is established there is probably a delicate balance between the needs of the growing body for the various B fractions and the synthetic powers of the bacteria. If an unfavorable environment depresses the activity of the organisms in the rumen the body must inevitably suffer a deficient supply of the B fractions.

Likewise, we are aware today of the numerous and specialized requirements of bacteria themselves for specific nutrients including the common vitamin B fractions. Therefore, the body of the ruminant may indirectly rely upon the food supply to furnish these B fractions because certain of them are essential for the growth of the rumen flora.

During the past decade a number of attempts have been made at Cornell to rear young ruminants, mostly lambs, upon synthetic diets supplemented with various mixtures of B vitamins. T. Shen (231) reared two lambs from birth upon synthetic diets in which yeast supplied the B fractions but these lambs grew poorly and remained at about two-thirds normal size when they were adults. All attempts at combinations of purified B vitamins have failed to produce normal growth in lambs fed synthetic diets (Rasmussen 207). Inasmuch as numerous studies have shown the B vitamins are synthesized in the rumen, the results of these lamb experiments must indicate that the medium provided by synthetic diets for the growth of the flora is unsuitable in comparison with that provided by natural feedstuffs. Possibly there are factors in feedstuffs that cannot be synthesized in the rumen. Johnson (121) was able to keep calves upon a synthetic diet from the 13th day until they were 130 days of age but usually these calves failed.

It seemed to make no difference in feeding Johnson's calves whether the synthetic diets contained vitamin B_1, riboflavin or the "grass juice" factor. All failed in time. The chief conclusion today is that a rather complex set of conditions must be understood before we can develop optimum conditions for supplying the calf with these fractions. Phillips *et al.* (201) indicate B complex vitamins will aid in relief from scours in young calves. They mention that niacin and pantothenic acid may be the fractions that are needed. This matter needs further careful study.

Yeast feeding. Various attempts have been made to determine if yeast provided nutrients not available to the calf in the usual ration of hay and grain. Eckles *et al.* (65) found no increase in the growth of calves from supplementing the usual diet with dry yeast. From the analysis of the yeast fed, they must have had a product with much extraneous matter. Newman and Savage (191) found favorable results from including 1 to 6.25 per cent of yeast in the mixture fed calves. They obtained better growth and better feed conversion from feeding diets containing yeast. The favorable results obtained by Newman and Savage were statistically significant, but when some of this work was repeated by Gardner (84) in 1940 the results were not so favorable. These apparent contradictions are probably the result of the interplay between the yeast and the bacterial flora.

From time to time calves have come to our attention on New York farms that were obviously malnourished although supplied with good hay and other feedstuffs. Upon examination these calves have been relatively free from parasites. For some reason the flora of the rumen seemed to fail to satisfy specific needs of these calves. Upon feeding such calves a suspension of dried yeast they responded in a few days by regaining their appetites for hay and grain. This matter has been discussed briefly (McCay, 166, Baker 10).

Bechdel (17) has reported feeding calves a B-deficient diet consisting of beet pulp, corn gluten meal, casein, rice, cane sugar, corn starch, hominy and mineral mixture. Rats suffered from symptoms of B deficiency on this ration but calves showed no symptoms when fed the diet from an age of 138 to 430 days. Three of the poorest were fed autolyzed yeast but the response was uncertain. Two cows in this study were placed upon the deficient diet low in vitamin B_1, two months before calving. The calves born were normal. A poor calf, however, seemed stimulated by feeding this yeast. This evidence indicates that the calf in most cases can satisfy its requirement for B_2 by rumen synthesis.

Recently, Wagner, Booth, Elvehjem and Hart (263) have shown that in a fistulated calf rumen, synthesis of thiamin, riboflavin, nicotinic acid, pantothenic acid, pyridoxine and biotin occurred on a diet of acid-washed casein, urea, corn starch, corn molasses, bleached wood pulp, salt mixture and cod-liver oil. The addition of 200 mg. of thiamin appeared to increase the synthesis of certain factors. No evidence for the destruction of thiamin in the rumen was found. Unpublished data from the Cornell Station indicate synthesis of thiamin, riboflavin and nicotinic acid when calves were fed a diet of vitamin-free casein, corn starch, sucrose, minerals, regenerated cellulose, cottonseed oil and vitamin A and D concentrate.

INORGANIC ELEMENTS

In studies with calves most attention has been given to Ca, P and Mg. Little is known about Na, K, Cl, F, I, Cu, Co and other minor elements.

Mitchell and McClure (180) have provided tables showing the requirements of growing cattle between body weights of 300 and 1200 pounds, for Ca and P. As the calf grows older within these ranges the feed Ca requirement drops from about 11 to about 5 grams per day while the P need remains at a level of about 10 grams. In 1917 Armsby (6, p. 415) summarized the daily requirements of calves for Ca and P from the literature then available. Thus at 18 days the calf stored daily in per cent of its body weight Ca, 0.02 and P 0.01 while at 150 days these values were 0.007 and 0.006.

When McCandlish (157) reported his attempts to rear calves upon a milk diet he observed their bones were poorly calcified and they were subject to fits before they died at about 200 days of age. In 1923 (160) this same author reported that grain failed to supplement the milk but that hay did. He thought the grain furnished too much Mg and too little Ca. At about this date there was considerable interest in Mg since Palmer *et al.* (197) found that feeding 156 grams of Epsom salts daily to a cow caused Ca to be lost from the body unless the diet was rich in P.

In 1927 Theiler *et al.* (248) found a heifer could make good growth upon a ration containing 10 g. of P and about 6 of Ca. Robinson *et al.* (211) found that different sources of Ca fed calves varied in retention. Thus, $CaCl_2$ caused a negative balance in the P. Ca lactate proved an excellent source of Ca while bone meal was intermediate in value between the lactate and the chloride. These authors estimated only 10 per cent of the Ca of the chloride was retained, 20 per cent of that of bone meal and 50 per cent from the lactate. These studies deserve further attention.

The extensive reviews of Theiler (247) provide excellent summaries of the needs of growing calves for Ca and P when vitamin D is not a factor due to the sunshine of South Africa. He noted that the Ca intake could be made much lower than the P without running into disease. He observes that no calf is born with rickets since the cow sacrifices her own bones in order to produce a normal calf. The undersized calves found at nine months of age on pastures deficient in P resulted from a lack of milk rather than because the milk lacked this element. Theiler claimed the rickets found in sheep were similar to those of calves and babies, while the analogous disease of goats resembled the osteodystrophy found in horses.

Emphasis is laid by Theiler upon rickets as the non-calcification of new tissues rather than the decalcification of bone. He says that ossification in the proximal bones takes place in the dog at the age of 18 months but not until an age of 3 to 4 years in herbivora. Likewise, he notes the cartilage of the vertebrae do not close until dogs are a couple of years old and horses, swine and ruminants are 4 to 6 years of age. He stresses the importance of such factors as being on their feet from an early age in the case of domestic animals.

Theiler *et al.* (248) fed growing heifers two years on diets low in P to simulate conditions found in pastures. They obtained the typical clinical picture resulting from lack of calcification of the cartilage characterized by (a) delay in development, (b) bending of extremities, (c) thickening of the epiphyses and (d) exposure of nerves leading to stiffness and lameness. To relieve pain, animals take unphysiological positions and become malformed. Finally, the skeleton becomes fragile and the animals get depraved appetites for old bones. These workers saw no tetany in their animals on diets low in Ca or P. This may have been due to the fact that their animals were often about a year old at the start of the experiment.

Huffman *et al.* (112) increased the interest in Mg when they found it exerted a sparing action on the need for vitamin D in calves. Feeding one per cent of $MgCO_3$ in their diets seemed to prevent calves from developing rickets. The workers also suggested that the reason calves developed tetany on whole milk diets was a deficiency in Mg. In calves dying in tetany they found very low Mg values in the blood but normal levels of Ca and P. Sjollema (235) challenged these conclusions and thought the tetany was due to the ratio of Ca to Mg.

Cunningham (42) found that restriction of Mg in the diet lowered this element only in the blood and bones. Increased dietary intake raised it only in those. Where cattle were subject to "grass staggers" Cunningham found the grass was normal and the grass hay had 0.3–0.4 per cent MgO. He found only the blood low in these cases of staggers but believed this trouble was cured by feeding magnesium salts.

Allcroft and Green (2) found seasonal variations in the level of Mg in the blood with a maximum in August and a minimum in December. In February the level was nearly as high as in June. In Michigan, Duncan *et al.* (58) found the values highest in November (2.55 mg. per cent) and lowest in July (2.26 mg. per cent).

In an experiment covering 255 days Knoop *et al.* (131) fed calves whole milk supplemented with 1 per cent of the dry matter of the milk as well as small amounts of iron and copper. The Mg level of the blood remained normal where this element was fed. The feeding of this element did not seem to modify the ash or breaking strength of the bones.

Much remains to be learned about the requirements of the calf for Mg. This seems to be higher than that claimed for other species. In Johnson's (121) studies a level of Mg was included in the diet similar to that usually fed rats but still his calves developed symptoms of deficiency. In the future, discussions of Ca and P will need to include Mg.

Bisschop *et al.* (28) found they could check the bone eating habits of South African calves by feeding bone meal. When the calves were 1 to 2 months of age they were given a half ounce daily. At six months of age this had been increased gradually to 3 ounces. At six months of age the calves fed bone meal had 6 mg. of inorganic P per 100 cc. of plasma while the others were below this and steadily declined. They concluded that up to a certain point a deficient skeleton can support normal body development but after this there is failure.

Few cases of areas deficient in Ca and P have been reported in America. Gullickson *et al.* (92) found such an area. These calves were suffering from a calcium and vitamin D deficiency. This became worse when they fed NaH_2PO_4 but better after they fed a daily supplement of 2 to 9 grams of $CaCO_3$. Under these conditions they estimate the calf may need less than 100 Steenbock units of D per day.

Meigs (179) concluded his excellent review with a description of the unfortunate results of discoveries concerning mineral deficiencies in animals. Unscrupulous individuals pretend these deficiencies are more widespread than they are and even make unwarranted claims for tests to detect the troubles. This is all done for the purpose of selling supplements at very high prices. The part calcium plays in the nutrition of the fattening calf has been discussed extensively by Weber *et al.* (261).

Sodium chloride. Very little information can be found about the requirements of calves for salt. This is usually left to the judgment of the calf. McCandlish (162) made some observations on the amount consumed. A calf usually ate about 0.01 lb. daily but at times it doubled this and at others ate only a fifth as much. On pasture the salt intake increased about 3 times. On a whole milk diet there was also a tendency to increase the salt eaten.

Theiler *et al.* (248) estimate that the salt need is not more than 1.5 grams of Na and 5 of Cl daily for growing heifers.

Trace elements. Knowledge of the importance of the trace elements in the diet of the calf is meager. Iodine has been discussed by Welch (265). He observed that if calves are not born hairless and dead they have a good chance of recovery even though the thyroids are much enlarged during the first weeks of life. The way to prevent this is to feed the cow some form of iodine either in her salt or drinking water during the gestation period. Mitchell and McClure (180) have given a good summary of the literature concerning iodine in live stock feeding. Welch (265) recommends the feeding of iodized salt to cows. If this salt contains 0.02 per cent he estimates the cow will get 0.3 grams in 5 months. Iodized salt lost its iodine in a few days if exposed to sunlight and rain but may lose only 10 per cent per year if stored under a shed according to Johnson and Harrington (120).

Kalkus (125) observed that as many as 70 to 80 per cent of the calves in certain areas in Washington had enlarged thyroids. If the springs were early this trouble subsided promptly but if the springs were late and snow lay on the ground calves were often lost. Some of the calf thyroids enlarged until they weighed 300 to 500 grams. Calves born in the autumn with enlarged thyroids had a better chance of living.

Rusoff (218) by spectroscopic estimation on four calves found fifteen elements in the tissues as follows: Al, Ba, B, Cr, Co, Pb, Mn, Mo, Ni, Ag, Sr, Sn, Ti, V and Zn. He was unable to detect any Sb, Be, Bi, Cd, Cs, La, Li, Th, W, Y and Zr. No differences were found between the normal calves and a "salt sick" one.

The so-called "salt sickness" of cattle which occurs on the sandy soils of Florida leached of mineral nutrients by the rainfall over many years, has been ascribed to a lack of various trace elements but the evidence has never been very clear-cut. In 1931 Becker *et al.* (22) recommended salt mixtures containing Fe and Cu. At a later date Neal and Ahmann (188) on the basis of limited experimental results concluded that Co was the essential needed in this disease. A small number of calves seem to grow all right with a daily supplement of 5 to 10 mg. of Co fed as the sulfate. However, these authors could get no test for Co from the hays that also seemed to produce recovery in these animals.

Only a few studies have been made to establish the requirements of the calf for Fe and Cu. Neal and Ahmann (188) found their calves failed upon a diet of Natal grass hay, corn, skimmilk, cod-liver oil and whole milk with supplements of Fe and Cu. Archibald *et al.* (4) found a nutritional anemia in Massachusetts which responded to Fe treatment.

Knoop *et al.* (132) ran an experiment for 255 days upon 12 Holstein calves to determine if milk supplemented with Fe and Cu could serve as a complete food. They lost only one calf. They used 40 to 60 mg. of Cu and 400 to 600 mg. of Fe fed as sulfate and oxide respectively, per day. Toward the end of the period the calves tended to develop rickets. The differences with and without supplements were not marked in either the number of red cells or the hemoglobin, being 8.6 and 9.8 for erythrocytes and 7.6 to 10.5 grams of hemoglobin. The higher values were found in cases of feeding the supplements. The Fe and Cu storage in the livers of calves fed milk alone was found to be 91 and 35 mg., while in calves fed supplements these values were 359 and 550. Whether or not these high levels of Fe and Cu are desirable in the liver from the possibility of catalytic destruction of fat soluble vitamins is unknown.

Milk as the sole diet of calves. Attempts to rear animals upon milk alone have given much information concerning the value of small amounts of inorganic constituents. Cannon (36) reviewed the early literature concerning whole milk diets and the development of rickets and tetany on such diets but offered no solution of the problems. Today these problems still remain unsolved although part of the deficiencies of milk are due to the level of the minor elements of copper and iron.

Milk products in the rearing of calves. Since very early times much attention has been given to skim milk. McCandlish (161) has provided a good review in this field. Wilson *et al.* (271) got fair growth feeding calves skim milk supplemented with flaxseed containing 30 per cent fat.

As early as 1904 Otis (196) claimed gains of 1.77 pounds per day for calves fed skim milk. He tried the addition of rennet just before feeding but found the calf did not improve when fed the clabbered milk. He also fed buttermilk and found calves grew nearly as well as upon skim milk. Eckles and Gullickson (63) also found buttermilk gave good growth with an absence of the usual troubles encountered in rearing calves.

Woodward (279) fed calves $\frac{1}{5}$, $\frac{1}{6}$, and $\frac{1}{7}$ of their body weight of skimmed milk. Even at the lowest level the calves made moderate gains of 0.95 lbs. per day. By drinking 80 per cent more milk they could make 50 per cent greater gains.

Cod-liver oil has been used to replace the fat of whole milk but in general this has not proved satisfactory. Cottonseed oil has not been as good as cod-liver oil. Whole flaxseed meal has been tried many times but it also gives poorer results when combined with skim milk than does whole milk. Corn meal, oat meal, ground peanuts and peanut meal have also proved only partly satisfactory. Even in the light of the limited knowledge of today it is apparent that these substitutes are deficient in the characteristic fatty acids of butter as well as vitamin A. No one knows whether these lower fatty acids of butterfat have a distinct part in the metabolism of the calf.

Skim milk powder can be fed to calves either dry or reconstituted with water. The latter seems better for very young calves. Skim milk powder has given good results in the hands of the following investigators: Savage and Tailby (222), Woodward (278), Bartlett (12), Hart *et al.* (103), Rupel (214), Crawford and Krauss (40), Lindsey and Archibald (145), Rupel and Bohsted (215), Savage and Crawford (224), and Williams and Bechdel (270).

Dried whey is a useful calf feed but seems slightly inferior to dry skim milk in the tests that have been made (Morrison *et al.* 183, 184; Rupel 216). Inasmuch as there is a considerable waste of whey, it may prove a useful substitute for dry skim milk during periods of scarcity of the latter.

Various buttermilk preparations have proved useful in feeding calves. Both dried buttermilk and semi-solid as well as the original have proved useful (Woodward 278, Knott 133, Idaho 115, Idaho 116, Rupel 215, Hart 103, Idaho 117, Idaho 118).

Many of the supplements for these dairy products have been tried because of availability rather than because they were sanctioned by a knowledge of useful components. Malpeaux (150) found sugar superior to starch. Molasses did not prove useful in the hands of Woodward and Lee (277).

A combination of starch and starch-splitting enzymes was approved by many workers (Gouin 87, Maswersit 154, Gouin and Andouard 88, Dolscius 51, Reichert 210, Pirocchi 200). Wellman (266) got unfavorable results with such a supplement.

Among the fats Wellman (266) found beef suet unsatisfactory. Peanut oil was found satisfactory by Peterson (199) and Fingerling (76). Palm oil also proved useful in the hands of Hangel (99). Possibly the calf utilizes these lower melting fats better than the higher ones such as beef suet. This is true for guinea pigs. The calf has never been studied in this respect. Furthermore, linseed oil has proved satisfactory for calves, Dornie and Daire (52).

Many plant products have been tested but none have given outstanding results (McCandlish 161). These have included corn meal, oats, oat meal, flaxseed, bran, rice flour, barley flour, wheat flour, ground rye, kaffir meal, and linseed meal. The many comparisons of the relative merits of these products have been conflicting. Better results can be anticipated when the requirements of the calf and the specific properties of the products are better understood.

CALF MEALS AND GRUELS

In the historical introduction some of the early feedstuffs used in gruels and meals were discussed. The theory of using a complex mixture of materials for feeding calves is based upon a realization of ignorance concerning the nutritional requirements. As the calf has its requirements more rigidly defined it should be possible to simplify mixtures to produce normal animals.

In 1894 Haecker (96) reared calves upon skimmed milk supplemented with flax meal, bran and corn meal. In 1902 Hayward (104) reported good results with some mixtures of feedstuffs fed as gruels. Thus, he fed a mixture of flour 1, flaxseed meal 2 and linseed meal 3. This was started when the calf was two weeks old and the gruel was made by adding 2½ pounds of the dry mixture to 2 gals. of boiling water. Another meal used by Hayward was wheat flour 30, coconut meal 25, dry skim milk 20, linseed meal 10 and dry blood 2. It is worthy of note that dry skim milk called "Nutrium" sold for ten cents per pound and dry blood sold for three. Dried blood was supposed to check the scours and wheat flour was also supposed to "keep the bowels from getting too loose." Both problems are still with us today. Possibly some of the substances rich in pectin may prove useful in feeding calves as they have in the case of dogs (McCay and Smith 171). Examples of such materials are the residues that are left after pressing juice from grapefruit, tomatoes and apples.

Another mixture tried by Hayward was corn meal 13, dry skim milk 20, flaxseed 1.5, dried blood 2, flour 30, coconut meal 6, and chopped oats, 6. Calves liked this mixture but did not do as well upon it as they did upon other mixtures.

In 1915 Lindsey (146) published a good review of the calf meals in use up until that date. In this review there are mentioned some familiar names such as Liebig's Calf Soup, Hansen's Potato and Barley Malt, Hayward's Calf Meal, Lactina Suisse, Bibby's Cream Equivalent, Blatchford's Calf Meal and Schumacher's Calf Meal. In these meals, he notes, were such ingredients as locust bean meal, wheat flour, linseed meal, flaxseed, coconut meal, ground beans and peas, cocoa shells, cottonseed meal, fenugreek, salt, rice polishings, starch, tapioca and sage. Some of these materials such as fenugreek with its high content of choline, rice polish with its B fractions, cocoa shells with possibly some D activity, interest us today due to recent discoveries.

Lindsey ran small scale tests with mixtures of corn meal, middlings, flaxseed meal, flour, salt, glucose, ground oats, blood flour, barley malt and potassium bicarbonate. He fed such a mixture as a supplement to skimmed milk. His calf meals were analyzed and the composition was the following: ash 2 to 6 per cent, fiber 1 to 6 per cent, protein 15 to 24 per cent and fat 5 to 12 per cent. In general the author did not favor cottonseed meal, fenugreek, anise or St. John's bread as ingredients of calf meals but did favor the commonly used ingredients as well as oat flakes and malted grains.

Carr *et al.* (38) studied the utilization of nitrogen from various combinations of feedstuffs as described previously. Their studies were run for periods too short to learn of possible vitamin or mineral deficiencies in the mixtures but they do show something of nitrogen storage. On a mixture of equal parts linseed meal, soybean meal, cottonseed meal and wheat middlings, about 27 per cent of the nitrogen was stored. On one of hominy feed, linseed meal, white flour and blood, about 32 per cent of the nitrogen was retained. Twenty-three per cent of the nitrogen was stored from a mixture of equal parts soybean meal, linseed meal, cottonseed meal, wheat middlings and dried blood. For a mixture of about equal parts hominy feed, linseed meal, wheat flour and casein, the storage was 30 per cent but for skimmed milk and a dried mash this storage increased to 40 per cent. They found the nitrogen about evenly distributed between the urine and feces in rations that produced good growth.

Caldwell (33) was well ahead of his day inasmuch as he studied not only such common protein sources as blood and grains but he also used clover juice in one of his studies. Today the literature contains the term "grass juice factor."

The meal devised by Maynard and Norris (155) was a mixture of corn meal 25 per cent, ground oat groats 15, red dog flour 25, linseed oil meal 15, ground malted barley 10, soluble blood flour 10, limestone 1, bone meal 1 and salt 1 per cent. This meal is still in use with the following changed composition: corn meal 20.75 per cent, flour 22, oat flour 15, linseed meal 15, malted barley 10, dry skim milk 12, blood flour 3, salt 1, bone meal 1 and cod-liver oil concentrate 0.25 per cent. This cod-liver oil concentrate contains 3,000 units of A and 400 of D per gram. For several years farmers in New York and adjacent states have used 900 to 1,000 tons per year. This means that 4,000 to 6,000 calves per year have been raised on it. This mixture has also proved useful for many years as a stock ration for white rats.

Ellington and Knott (70) report good growth upon a mixture of corn meal 40 per cent, alfalfa leaves 20, wheat bran 20 and linseed meal 20 per cent. Davis and Cunningham (49) used a mixture of corn meal 2, wheat middlings 4, oat groats 2, linseed meal 1, blood meal 0.5, bone meal 0.2 and salt 0.2. This was fed as a gruel. Berry (26) used a mixture of ground oats 1, bran 1, corn meal 1, dry skim milk 1, linseed meal 0.5 and salt 1. He had some trouble with this mixture fed as a gruel.

Morrison and Rupel (185) fed a mixture of corn meal 25, wheat feed 25, malted grain 25, linseed meal 12, blood meal 10 and salt 1. Some bone meal was also used. They found no improvement in this mixture for calves from the addition of 10 per cent tankage. Bender and Perry (25) used a mixture of corn meal 100, ground oats 150, wheat bran 50, linseed oil meal 50, soluble blood flour 50, bone meal 4, limestone 4 and salt 4. They state that blood meal or dried blood cannot replace the soluble blood flour. When the calf reaches 6 months of age they change the composition to equal parts of corn meal, ground oats and bran with 0.3 part of linseed meal.

Archibald (5) made some attempt to improve calf meals from the standpoint of physical composition. He ground some in a ball mill and added gelatin to support a suspension. He also tried cooking the meal. Digestion trials indicated little difference except that the cooked dry matter seemed a little better utilized. In general the values for utilization of the cooked and raw were dry matter 75.72 per cent, protein 74.78 per cent, and ether extract 95 per cent. These utilization values are very similar to those for dogs fed dry feeds composed largely of breakfast food by-products with the protein provided by meat and milk (McCay 165).

In recent years attention has been given to the improvement of calf meals by making substitutions. Elting and LaMaster (71) found a mixture of about 40 corn meal and 40 ground oats could be supplemented with 10 parts of fish meal, skim milk or cottonseed meal. Double this amount of cottonseed meal was fed in one trial. Huffman (113) also found that cottonseed meal could be used if the remainder of the rations was complete. Cunningham and Addington (43) were able to feed calves cottonseed meal but their calves showed the signs of deficiencies, such as chewing wood.

Campbell (35) found he could supplement a mixture of equal parts of corn, oats and bran satisfactorily with either linseed meal or fish meal. In a study using Holstein calves Krauss *et al.* (135) found that blood meal could be replaced by skimmed milk, fish meal or meat scrap. Williams and Bechdel (270) found skim milk and blood meal of equal value.

Soybeans in different forms have been studied by Hilton *et al.* (107). In a mixture of equal parts of corn, oats and bran they found ground raw soybeans as good as linseed oil meal. Hilton *et al.* (106) found no advantage in grinding their meals except that calves consumed about 10 per cent more. Skinner and King (236) attempted to improve soybeans by roasting them but their results were negative. Shoptow (233) attempted to rear calves upon soybean milk made by suspending a pint of flour in nine of water. Calves did not seem to thrive as well upon this milk as the babies of China that were fed by Tso.

The calf seems to be able to utilize rather diverse mixtures of plant products. In the popular bulletin of Herman (105) are recommended mixtures varying from 30 to 50 per cent in corn meal, from 25 to 30 in ground oats, from 20 to 50 per cent in wheat bran and from 10 to 50 per cent in linseed oil meal. Perhaps the calf survives in spite of this mixture.

Willard (268) has found the greatest consumption of hay results from keeping the available grain low from a fairly early age. He found the amount of hay consumed bears almost a direct relation to the age of the calf.

A few special feedstuffs for dairy calves have been given a little study. Calloway (34) found he could use blackstrap molasses, starting to feed about 2 ozs. daily when calves were 3 weeks old, and increasing this gradually to 2 pounds daily when they reached 24 weeks. In general the use of molasses seems limited during the early months of calf feeding. Cocoa meal was tried by Ellenberger and Aplin (69). They found calves disliked it and there was even some evidence of injury.

CALF STARTERS

In recent years the trend is away from calf meals and gruels to calf starters. The calf starter method of raising calves as outlined by Turk (255) consists of feeding a minimum amount of whole milk (350 lbs.) for a short period (7 to 10 weeks). The calf is then weaned and must grow after weaning on a diet of mixed ingredients, a calf starter, hay and grain. The calf starter is fed *ad lib.* as soon as the calf will eat it until it is 12 weeks of age, and then the amount of calf starter is limited to 4 lbs. per day and a simple mixture of ingredients like ground corn and oats, wheat bran and linseed oil meal is added. At sixteen weeks the calf starter is dropped from the ration because of its cost. Hay is fed *ad lib.* as soon as the calf will eat it and water is kept before the calf at all times.

To Mead, Regan and Bartlett (176) should be given the credit of beginning in 1924 the feeding of calf starter dry with a minimum of whole milk. This was an epoch-making change because it meant doing away with liquid feeding except a small amount of whole milk. Thus, the labor of raising calves has been greatly reduced.

Bender and Bartlett (24), Bender and Perry (25) and investigators at several experiment stations besides New Jersey explored further the value of the New Jersey calf starter.

Savage and Crawford (223) developed the first Cornell dry starter in 1933. These starters have been improved to the formula given in the summary of this review.

In New York, New Jersey and northern Pennsylvania more than 1,000 tons of calf starter are sold by one company each year. This means that more than 6,000 calves are raised on this starter alone each year. The use of the dry calf starter method is now (1942) much greater than the use of calf meal gruel method. Many commercial closed-formula calf starters are sold in pellet form. Newman and Savage (191) could see no advantage in the use of pellets. Their investigation of this point, however, was not extensive enough and should be repeated. In our opinion the calf starter method of raising calves is the most economical. Calf starters will be improved and their cost per ton lessened as we find out more about the real needs in a calf's diet.

Special studies with dairy calves. In recent years interest in the selection of proper feed mixtures by growing animals has been revived due to evidence that babies and rats can select adequate diets if allowed free access to a number of ingredients. From time to time such trials have been made with calves and swine but the results have usually been unfavorable. McCandlish (162, 163) made such studies. From them he concluded calves prefer whole corn and oats to the ground grains. He found they did not like hominy feed and did like linseed oil meal better than bran or corn gluten. In general his calves selected rations with a narrow nutritive ratio. They also ate considerable amounts of both charcoal and salt. One can understand the salt consumption but is rather mystified by the charcoal.

Olson (194) found calves in self-feeding trials could not balance the ration and showed signs of rickets in about three months. Among 20 calves the range in feed eaten was very great. The following are some of his values in round numbers: yellow corn 3 to 723, white corn 4 to 405, wheat bran 2 to 309, linseed oil meal 11 to 652, oats groats 7 to 368, whole oats 22 to 682 and alfalfa 1 to 192. These calves were capricious about their selections. Self-feeding of balanced rations proved satisfactory to Nevens (190).

The old question of the possible unfavorable effects of milk foam upon the dairy calf has been studied from time to time. Olson (193) fed one group of calves twice as much foam as occurs normally and another none. He found no injurious effects. Cannon, Espe and Shultz (37) also studied this problem and concluded there were no ill effects from feeding the foam. Tretsven and Keyes (253) came to the same conclusions. On the other hand, McCandlish (161) concluded that foam on mechanically separated milk produced digestive disorders in calves.

WATER CONSUMPTION

McCandlish (161) concluded water should be allowed calves even when they are getting milk. In studying mature animals Kellner and Kohler (128) found from 2.7 to 4.2 pounds of water were drunk per pound of dry feed at a temperature of 16–17° C. Under such conditions 46 per cent of the water was in the feces and 29 per cent in the urine.

McCandlish (163) found a daily consumption of 4 pounds of water during the first month and 8 pounds per day during the second. Morrison *et al.* (184) found calves made better gains when allowed water even if they were being fed skimmed milk. For calves receiving liquid milk Atkeson *et al.* (9) found water of little importance for the first 8 weeks. They found a calf at 4 weeks of age might drink only 10 lbs. of water per week and when 25 weeks old might have increased this to 270 lbs. Elting and LaMaster (71) advocated warm water allowances for calves after weaning to accustom them to water drinking.

GROWTH OF CALVES

The rate of growth of calves has always been of much interest to nutrition students because a deficient diet is most easily detected in a slow growth rate. At the same time, however, the purpose of rearing calves is to produce cows with bodies of a type that will permit the optimum production of milk during a long lifetime. The possibility exists that the optimum lifetime performance may not be the result of the maximum rate of attainment of adult body size although most nutrition workers have assumed this to be true for all animal species. Some breeders of Guernseys and Jerseys prefer a slower rate of growth since they feel it produces a better cow. However, these breeders are as deficient in data to support their point of view as are those who try to produce the greatest possible growth rate in developing calves. This whole problem has been discussed in more detail elsewhere (McCay and Crowell 173, McCay 172).

The greatest need today is for establishing the interrelationships between the diet of the calf, the rate of growth, the diet of the cow and the lifetime performance. In general the philosophy that has stimulated producers to promote the maximum growth rate in the calf is based upon the thesis that this method produces the largest and best formed cow. It is well known that calves and sheep retarded in growth as the result of rations inferior in quality and amount are likely to be permanently stunted if this retardation is long continued. The first person to give serious attention to this problem in the case of calves was H. S. Waters (260).

Waters found that a constancy in body weight of calves may mean that the skeleton continues to grow while other parts such as the muscles may decline. By permitting periods of alternate growth and retardation he found a tendency for the body to compensate by growing more rapidly after periods of retardation. He concluded that the animal could grow steadily from birth to maturity, could store fat for bad periods, could prolong the growth period if retarded, could increase the growth rate above normal after a period of retardation and on a low plane of nutrition could conserve nutrients possibly by better utilization. There is need today for the repetition of Waters' research and the study of the animals throughout life.

Eckles and Swett (64) confirmed Waters' findings of a longer growth period from feeding scanty rations. They found little relation between the ultimate size of an animal and the weight of the calf at birth. They also found compensation in the form of increased growth rate after periods of retardation. They found heifers restricted in diet never became quite as large as others.

One of the best known growth studies was that of Eckles (61) in which he found the growth rate for 26 Ayrshire heifers from birth to 3, 6 and 12 months of age to be 1.12, 1.21 and 1.08 pounds per day respectively. Later McCandlish (159) reported corresponding figures for heifers of the four dairy breeds of 1.07, 1.37 and 1.17. In general McCandlish (161) stated that dairy calves should gain more than a pound per day during the first three months and in excess of a pound and a quarter during the following 6 months.

Since the extensive series of bulletins published from Missouri by Eckles, Ragsdale, Brody and many others, especially in the series "Growth and Development" beginning in 1926, give a comprehensive picture of growth not only in calves but in many other species, little space will be devoted to this very involved subject here. Ragsdale *et al.* (204) show that growth continues at a constant percentage rate (30 per cent per month) until the end of four months. Following this age in calves the growth rate declines at 4.5 per cent per month. The more recent discussion of the changes in organ weights in relation to body weights of animals is found in the bulletin of Brody and Kibler (31). This subject is of great importance since the relation of growth of parts to the growth of the whole body may be profoundly modified by different states of nutrition. The development of these parts in turn may have great influence upon the ultimate shape of the animal's body, the productive capacity of the body and the resistance of the animal to disease. A bare glimpse into the possibilities in this field can be seen in the recent reports concerning retarded growth (Pomeroy 203, McMeekan 175, McCay 172). Growth is far more than the increase in the body weight of an animal from day to day. It involves the changes in the numerous parts of that body. These in turn are the links in the chain that determine the capacity of the body to live and to produce.

Davis and Willett (48) could find no relation between the rate of growth of calves up to 2 years and the milk or fat production in the first lactation or the lifetime average of lactations. However, the factors that condition the rate of growth are very numerous and these need to be considered in such work. The total increase in the body weight upon one diet may mean different internal conditions than from another although the body weight increases may be identical. The internal structures of the body determine its productive capacity and only upon the assumption that gross body weight always provides a constant internal relationship of organs and the composition of these structures would we expect to find close correlations between the increase of the whole body and of the calf and productive capacity of the cow.

The calf as a converter of matter. From very early times the relation between the rate of growth and the efficiency of conversion has been clearly recognized. Thus, Stewart (243) comments on the profits from a calf that increased in body weight by an average in excess of 3 pounds daily for nearly a year. He states "from extra food comes all the profit." His discussion concerns meat production and not lifetime activities in producing milk and calves.

Hunziker and Caldwell (114) kept very careful records upon the food consumed by 10 calves for 26 weeks. With one exception all these calves gained between a pound and a pound and a half daily. About 4.5 pounds of dry matter were fed daily to produce these gains.

Armsby (6, p. 712) estimated that exclusive of the maintenance requirement a calf during the first month of life required 1.17 therms of net energy per pound increase. As the calf became older and the rate of growth declined this value increased to 2.29 therms during the 9 to 12th month. When the calf weighed 150 pounds he estimated the net energy requirement per day was 1.69 therms while at a body weight of 500 pounds this value was 3.78 therms.

Armsby and Moulton (8) estimated that calves had a gross efficiency of

9 to 10 per cent in the conversion of feed into body tissues. Ragsdale *et. al.* (204) have discussed this conversion efficiency and presented data showing the variability found when working with small groups of different breeds. Their values for dry matter consumed per pound of gain vary from less than 1 to nearly 20. As a rule their values increase from about 2 when the calf is a month old to 7 or 8 at the age of 11 months.

Purified diets for calves. In a preliminary report Johnson, Loosli and Maynard (122) indicated that only moderate success was achieved in rearing dairy calves from birth to 6 months of age on highly purified diets. At 48 to 60 hours after birth and after the calves had received colostrum they were changed abruptly to an artificial mixture of casein, lactalbumin, sugar, butter or lard, minerals and water that was similar in composition to cow's milk. The calves were given free access to a dry meal made up of casein, starch, sugar, cottonseed oil, cellulose and minerals and they were changed entirely from the artificial milk to the dry diet at 3 months of age. Under these conditions of feeding the growth rate of the calves was never more than 80 per cent of normal for the sex and breed. The addition to the basal diet of supplements of thiamin, riboflavin, yeast or grass juice did not uniformly influence the growth rate or general appearance of the calves.

In the initial studies certain calves developed magnesium deficiency and died in tetany. The addition of 25 to 30 mg. per kg. body wt. of Mg as $MgCO_3$ maintained the serum Mg at a normal level and prevented tetany. Some of the calves developed paralysis of the legs that continued to appear after the Mg intake was increased.

Subsequent studies by Loosli in 1941 (unpublished) have shown little consistent advantage in the rate of growth when 6 per cent of liver was added to the diet. While yeast supplements did not improve the growth rate their removal from the diet caused decline in the appetite and eventual failure in two cases. A number of calves have been successfully reared to 5 and 6 months of age. When they were turned to pasture or placed upon hay and grains they have made good growth and developed into apparently normal animals. While it appeared that many of the calves could have been continued for considerably longer periods on the purified diets their frequent failure to make normal increases with age in the feed intake and the occasional death of animals due to a specific leg paralysis clearly indicate that the diets studied are deficient for normal growth and development.

SUMMARY

Ragsdale (206) has given us growth standards for dairy cattle. His figures are given as standard weights at the end of each month. We have constructed table 4 from his tables to show standards from birth to 16 weeks of age by weeks. From data collected at Cornell in the investigations with different calf starters we show standard weights that may be expected when

TABLE 4

Standard weights for calves 1 to 16 weeks of age

Age	Ayrshire		Holstein		Guernsey		Jersey	
	Ragsdale	Cornell	Ragsdale	Cornell	Ragsdale	Cornell	Ragsdale	Cornell
No. of animals used	124–101	20	239–185	30	108–82	18	173–138	14
Birth	72.00	76.65	90.00	95.46	65.00	67.61	53.00	51.78
1 week	75.97	79.00	95.13	95.26	67.80	66.72	56.27	53.00
2 "	79.93	81.00	100.27	99.60	70.60	69.33	59.53	56.14
3 "	83.90	86.30	105.40	104.93	73.40	73.67	62.80	58.14
4 "	87.78	93.35	110.53	113.50	76.20	78.22	66.07	63.28
5 "	93.84	101.80	117.81	121.16	81.03	84.44	70.71	69.21
6 "	100.61	110.05	125.94	131.03	86 68	91.28	75.90	76.14
7 "	107.39	120.50	134.06	141.73	92.32	99.22	81.10	83.00
8 "	114.16	130.10	142.19	152.66	97.97	108.16	86.29	90.50
9 "	121.60	140.75	151.00	164.20	104.70	117.33	92.07	99.50
10 "	130.70	149.95	161.50	177.50	111.30	127.61	99.30	107.78
11 "	139.80	159.70	172.00	189.86	118.53	136.61	106.53	116.92
12 "	143.90	169.85	182.50	203.00	125.79	146.22	113.77	124.35
13 "	158.00	181.15	193.00	216.10	133.00	156.50	121.00	130.57
14 "	167.03	191.75	204.29	230.06	142.03	166.55	129.35	139.57
15 "	176.06	203.65	215.58	244.33	151.06	177.50	137.71	149.64
16 "	185.10	215.45	226.87	258.33	160.10	189.77	146.06	159.64
Ave. daily gain birth to 16 weeks	1.010	1.239	1.222	1.454	0.849	1.091	0.831	0.963

calf starters are used. As shown by the figures at the top of the columns Ragsdale averaged various numbers of calves to get his weights. For example, he had from 101 to 124 Ayrshire calves in his groups. Our weights are for calves fed formulas C S, M A Y, and similar formulas, with hay from birth to 16 weeks. This table shows that the calf starter method will grow calves somewhat heavier than Ragsdale's calves. This table will be valuable for comparison in future investigations.

From our review we cannot determine that a record of the height at withers is a better criterion of judgment as to the progress of a calf than a live weight record. Perhaps both should be kept. Both together are probably better than one alone.

In table 5 we suggest three formulas for a calf starter. The C S formula has been used commercially with more than 15,000 calves and has given very fine results. It is fed with a minimum of 350 lbs. of whole milk fed over a period of 7 to 10 weeks and hay and grain according to the method given by Turk (255).

Formula M A Y is designed as a cheaper formula to use less expensive ingredients. Alfalfa leaf meal is used as a source of A. Tomato pomace is a source of A, E, and pectin, irradiated yeast is used for D. This eliminates the need for cod-liver oil concentrate. Bone meal and dicalcium phosphate may be used interchangeably as a source of calcium and phosphorus. Unpublished results with 22 calves at Cornell show that this M A Y formula will give as good results as those raised at Cornell on formula C S.

TABLE 5

Calf starter formulas

	C S	M A Y	L O Y
	lbs.	*lbs.*	*lbs.*
Yellow corn meal	647.5	369.75	389
Rolled oats	560		
Crushed oats		400	400
Wheat bran	200		360
Heavy wheat feed		300	
Linseed oil meal	100	200	400
White fish meal	60	100	
Dried skimmilk	200	100	
Corn gluten feed	120		
Peanut oil meal		100	
Cocoanut oil meal		100	
Corn oil meal	40		
Molasses		100	160
Alfalfa leaf meal		100	100
Tomato pomace		60	60
Brewers' yeast	40	40	100
Irradiated yeast		0.25	1
Ground limestone	10	10	10
Steamed bone meal	10	10	
Dicalcium phosphate			10
Salt	10	10	10
Cod-liver oil (conc.)	2.5		

The L O Y formula is an attempt to furnish all the known needs of calves from simple well known ingredients to the 16th week when fed with 350 lbs. of whole milk and good hay, green enough to furnish a goodly amount of A and D. Formula L O Y promises well.

Computation shows that formulas M A Y and L O Y will furnish enough energy, protein, vitamins and minerals after the fourth week when calves begin to eat considerable starter and hay. From birth to the end of the fourth week we recommend the feeding of colostrum for the first week and extra amounts of A and D and perhaps some of the fractions of B according to the methods of Phillips *et al.* (201). It is of no use to put more vitamins into the starter because the calves do not eat enough of it and hay to provide these things from birth to the fourth week.

We have written this summary in this concrete form to show what we have gathered from the literature and data to date (1942).

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PHOSPHOLIPIDS IN DAIRY PRODUCTS. I. DETERMINATION CHOLINE IN MIK FAT[1]

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INTRODUCTION

Although they are present only as a minor constituent of milk, the phospholipids have received considerable attention in dairy chemistry, since they are regarded as having a part in the development of certain off-flavors in dairy products. The substance trimethylamine has long been considered to be formed by the oxidative deterioration of lecithin in butter and thus to give rise to a fishy flavor (9). Of further interest is the view that the phospholipids may undergo oxidation in sweet cream butter and thus cause the development of stale storage, bitter or metallic flavors (1), as well as other oxidative defects in other dairy products (4, 20, 24). They are also of interest due to their emulsifying, water-binding and other colloidal properties (6, 11, 17, 18).

Data on the quantitative determination of the total phospholipids have been based, for the most part, on the microchemical determination of phosphorus in extracted milk lipids. In recent years, methods have been developed for the microchemical determination of choline, which makes up one part of the lecithin molecule. It has been the object of the present work to apply one of these methods to the estimation of choline in fat extracted from dairy products.

REVIEW OF THE LITERATURE

The older work on the phospholipids and their structure is reviewed in the monograph of McClean and McClean (13); a more recent work is that of Thierfelder and Klenk (21). The review of Working and Andrews (26) has also recently appeared. For the present work, the facts that lecithin gives choline, fatty acids, glycerol and phosphoric acid on being split, and that cephalin gives the same residues, except that ethanolamine is said to

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appear in place of choline, and that spingomyelin is said to give sphingosine, choline, a fatty acid and phosphoric acid, are of interest.

In the extensive literature on the subject of phospholipids in milk and its derivatives, it was found that Müller (15) had reported choline and methyl guanidine in milk, and that Tolkachewskaya (25) isolated 0.36 gram of choline chloroplatinate from 20 liters of milk. Bischoff (3) obtained choline chloroplatinate from phospholipid material, which was prepared from extracted milk lipids. Osborne and Wakeman (16) obtained the compound in a similar manner. Diemayer, Bleyer and Ott (7) used the Roman (19) method to determine choline in milk phospholipids, which they prepared from lipids extracted from whole milk powder.

The micromethods for the determination of choline which were of interest in this investigation, were the periodide method of Roman (19), and later modifications of this by Erickson and co-workers (8), Beattie's colorimetric method (2), and the method of Thornton and Broome (23) which may be of more value in further work. Reineck's salt was used in this investigation for qualitative purposes, except for a few trials with the method of Beattie (2), and a trial of the method of Kapfhammer and Bischoff (12) with solution of choline chloride.

In preliminary work it was found that periodide precipitates could be obtained from extracted milk fat hydrolyzates. The milk fat was extracted by the Mojonnier method. It was then boiled with 5 N sulfuric acid. The aqueous and fat phases were separated, and washings were concentrated on the water bath to 25 or 50 ml. With such hydrolyzates, precipitates were obtained with Roman's solution and Reineck's salt. Reproducible results were obtained with duplicate samples of milk fat by the Roman method. On the basis of these facts, the use of this method for the determination of choline in extracted milk fat was investigated.

EXPERIMENTAL PROCEDURES

The procedure for the determination of choline, as used in this investigation, was as follows: a 3 to 5 ml. aliquot of the neutral or slightly acid hydrolyzate or solution, containing not more than 1 milligram of choline, was placed in a 15 ml. glass-stoppered centrifuge tube. It was treated with 0.3 ml. of the Roman reagent (157 grams of iodine and 200 grams of potassium iodide in 1 liter of water per ml. of hydrolyzate). The sample was centrifuged for 3 minutes at a speed of at least 2500 r.p.m., and preferably 3,000 or more r.p.m. (lower speeds were not satisfactory). The tubes were then placed in ice water for at least 30 minutes. The thorough cooling of the tubes before filtering and washing the choline periodide was very important, and conditions had to be maintained at near the freezing point for good results. After the cooling, the excess precipitating reagent was poured off through filter paper on a chilled 16-mm. Buchner or Hirsch funnel. The precipitate was washed rapidly with five or six 2-ml. portions of ice-cold water until free from iodine solution.

The filter paper was returned to the centrifuge tube and the precipitate taken up in 3 ml. of chloroform. The chloroform solution of iodine was titrated with 0.01 normal sodium thiosulfate solution until the pink iodine color disappeared. A drop of starch indicator was also used.

Since the factor as used by Roman to calculate the weight of choline from the titration values obtained with thiosulfate was found by others (22) to yield only about 90 per cent of the choline actually present by weight, it was desired to derive a factor in order to arrive at a basis for calculating results. In each of two different trials, stock solutions of pure choline chloride were used. The choline chloride was recrystallized three times from absolute alcohol and dried in an Abderhalden drier at 84° C. The weighed bulb containing the salt was sealed immediately on being removed from the drier, then weighed to obtain the amount of choline, and finally broken in a beaker of water. The water was made up to volume, so that the weight of choline chloride per ml. was known. This was the stock solution and contained approximately one milligram of choline chloride per ml. To test the Roman method, this stock solution was diluted ten times before use. Several determinations were made by the method and enough choline was present in all trials to give a titration value of from 3.2 to 4.5 ml. of 0.01 N sodium thiosulfate.

The figures in table 1 represent milligrams of choline per milliliter of stock solution, as determined by the various methods.

TABLE 1

Comparison of the Roman method with various other methods for the determination of choline in dilute choline chloride solutions

Trial	Weight of choline per ml.	Calculated* from total nitrogen determination	Calculated† from weighed chloro-platinate	Calculated from Roman method	Per cent recovery by Roman method
1	0.125	0.130	0.128	0.122	95.3
2	0.107	0.103	0.106	0.098	93.3

* Total nitrogen was determined on the concentrated stock solution by the Kjeldahl method.

† The chloroplatinate was obtained from the concentrated stock solution and determined according to Thierfelder and Klenk.

The results obtained on the standard choline solutions by the direct weight, total nitrogen and chloroplatinate methods showed good agreement. The periodide method gave an average of 94.3 per cent recovery of the amount of choline present, when the factor as recommended by Roman was used.

Thus the factor, 0.1335 ÷ 0.943 or 0.1415 milligrams of choline was taken as equivalent to one milliliter of N/100 sodium thiosulfate solution, in this investigation.

For example, if a 3.69 gram sample of extracted milk fat required 1.92 ml. of 0.009 N thiosulfate to titrate the choline periodide from a 1/10 aliquot of the hydrolyzate, then, assuming the lecithin to be stearyl-oleyl lecithin, and to have a molecular weight of 806, and with choline having a molecular weight of 121,

$$\frac{1.92}{1000} \times 0.1415 \times \frac{0.009}{0.010} \times 10 = 0.00245 \text{ grams of choline in the sample.}$$

Then,

$$0.00245 \times \frac{806}{121} \times \frac{100}{3.69} = 0.442 \text{ per cent of lecithin in the sample.}$$

As carried out at first, the usual procedure for lecithin hydrolysis (14) was followed with extracted milk fat. This involved boiling the fat with 5 N sulfuric acid for 15 hours and subsequent removal of the acid as barium sulfate. It was desired to simplify these conditions as much as possible, in order to shorten the time for a determination.

An egg phospholipid preparation was made up according to the procedure of Bull and Frampton (5). This material had the following analysis: 3.61 per cent phosphorus, 1.73 per cent nitrogen, 10.5 per cent choline (Roman method); iodine number 68.5. This was dissolved in chloroform to give a solution containing 4.2 grams of the preparation per 100 ml. This was stored in the refrigerator in the dark. Sweet cream was then churned and the butter melted to obtain a butter oil. To 958 grams of the oil were added 100 ml. of the chloroform-phospholipid solution. The $CHCl_3$ was removed in a Claissen flask at 60 to 70° C. in a stream of nitrogen. The Mojonnier test showed 99.5 per cent of fat to be present. The mixture was then analyzed for phosphorus (10) and choline. The results are shown in table 2.

TABLE 2

Analysis of butter oil-phospholipid mixture for choline

Fat	Sample grams	Aliquot of hydrolyzate	Ml. of 0.0090 N thiosulfate	Mgm. choline present	Per cent choline in fat	Per cent lecithin in fat
Mixture	10.0	3/25	4.71	5.00	0.050	0.33
Mixture	10.0	3/25	5.00	5.30	0.053	0.35
Butter oil ...	20.0	3/25	0.15	0.18	0.0009	0.006
Butter oil	20.0	3/25	0.16	0.18	0.0010	0.006

The amount of choline in the fat, as calculated, was 0.046 per cent. The results for phosphorus showed that 0.0158 per cent phosphorus was added to the butter oil and that in two trials 0.0159 and 0.0150 per cent were recovered. This corresponded to 0.41, 0.41 and 0.39 per cent phospholipids respectively, in the oil. Blank determinations on the oil showed 0.005 per cent phospholipids.

The experiment was repeated with 497 grams of butter oil and 50 ml. of the chloroform-phospholipid mixture. The amount of phosphorus found averaged 0.0153 per cent; by calculation, 0.0153 per cent. This mixture was then subjected to hydrolysis with hydrochloric acid for various lengths of time. The amount of fat mixture used was 10.0 grams and 25 ml. of each strength acid were used. After the hydrolysis, the hydrolyzate was filtered off, the fat washed, the excess acid neutralized, and the hydrolyzate and washings evaporated to less than 50 ml. in the water bath. The hydrolyzate was then cooled, made to volume and tested for choline, as shown in table 3.

TABLE 3

Effect of variation in hydrolysis conditions on the analysis for choline

Strength of HCl used	Time of hydrolysis in hours	Aliquot of hydrolyzate used	Ml. of 0.0134 N thiosulfate	Mgm. choline found
5 N	3	6/50	2.46	3.8
5 N	10	6/50	2.60	4.1
5 N	18	6/50	2.55	4.1
1 N	3	6/50	2.68	4.3
1 N	10	6/50	2.75	4.4
1 N	18	6/50	2.60	4.1
N/2	3	6/50	2.70	4.1
N/2	10	6/50	2.77	4.4
N/2	18	6/50	2.64	4.2

The blank was 0.17 mgm. and the amount of choline added was 4.45 milligrams for 10 grams of fat. When weighed out, the fat contained 1.7 per cent of solvent. Thus, recoveries of 84 to 95 per cent were obtained, the average being 91 per cent. A recovery experiment was also made using lecithin prepared by the cadmium chloride precipitation method, with similar results.

The recovery of choline added as choline chloride to butter oil, with subsequent carrying through of the hydrolysis procedure was then undertaken.

A number of other trials were made using different stock solutions. The general average was 96 per cent recovery of choline. It was noted however, that it is necessary to have thorough cooling of the periodide precipitate or the losses become greater than 5 per cent. It was also noted that the amount of choline present should not give a titration value greater than 8 ml. of 0.01 N thiosulfate (22), otherwise too high results may be found.

In another set of trials, recovery of choline from mixed, slightly acid hydrolyzates from extracted milk fat was carried out to see whether or not there was any substance present which would interfere with the results.

In the first trial, 3 ml. of the hydrolyzate plus 3 ml. of standard choline required 3.88 ml. of thiosulfate. Three ml. of the hydrolyzate had originally required 1.99 ml. thiosulfate and 3 ml. of the standard choline solution required 1.99 ml. of thiosulfate. This showed a recovery of 97 per cent. In the second trial, 5 ml. hydrolyzate required 2.51 ml. thiosulfate; 5 ml. standard choline required 2.94 ml. of thiosulfate, and 5 ml. hydrolyzate plus 5 ml. choline required 5.55 ml. thiosulfate. This recovery was then 102 per cent. It was concluded that there was no interference with choline periodide precipitation in these instances. It is to be noted, however, that a clear solution must be had for the best results.

The extent to which duplicates agree in the proposed method of analysis for lecithin in extracted milk fat is shown by the following data for samples of milk, the fat for hydrolysis being obtained by the Mojonnier extraction method. The blank was taken as 0.05 mg. choline. The blanks found with butter oil hydrolyzates were from 0.05 to 0.20 ml. of 0.01 N thiosulfate.

TABLE 4

Determinations of choline and lecithin in fat from samples of whole milk

Sample cow	Fat test per cent	Grams fat hydrolyzed	Ml. 0.0102 N thiosulphate for 1/5 aliquot	Mgm. chloline in sample	Per cent lecithin in fat
58 A	3.74	4.24	1.90	1.36	0.21
58 A	3.74	4.24	1.75	1.27	0.19
58 A	3.74	4.20	1.88	1.36	0.21
58 A	3.74	4.20	1.93	1.40	0.21
450	5.07	4.12	1.94	1.52	0.24
450	5.07	4.12	1.96	1.42	0.22
450	5.07	4.16	2.10	1.52	0.24
450	5.07	4.16	2.15	1.56	0.24

Usually a 3 to 5 gram sample of extracted fat gave satisfactory results when boiled with 20 to 30 ml. of normal or half-normal HCl, with subsequent concentration of the hydrolyzate and washings to 25 ml.

DISCUSSION OF RESULTS

A number of experiments were made to determine the consistency of results obtained when extracted butterfat was subjected to relatively mild acid hydrolysis and the choline determined in the neutral or slightly acid hydrolyzate by a method which was modified slightly from that of Roman. The values obtained were consistent under the proper conditions, although the choline periodide is unstable at temperatures much above the freezing point. It was further found that in agreement with other work (22, 23), the factor which was recommended by Roman does not account for all the choline present in aqueous solutions. A corrected factor was used.

Tests made with hydrolysis procedure showed that aqueous normal hydrochloric acid was suitable as a hydrolysing agent for the splitting of the choline from the lecithin in the extracted fat. A three to five gram sample, or more, of fat was used.

Choline, which was added as freshly prepared egg phospholipids to butter oil which was low in phospholipids, was recovered to the extent of 91 per cent by the procedure. The recovery of choline added as the chloride to butter oil, and to hydrolyzates from extracted milk fat averaged 96 per cent.

SUMMARY

A modified Roman micromethod has been found applicable to the analysis of extracted milk fat for choline. A method for the determination of choline-bearing phospholipids in dairy products was thus found.

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A NEW COLORIMETRIC METHOD FOR THE DETERMINATION OF FREE FATTY ACIDS IN MILK FAT

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It has been recently shown (3) that the free bases of a number of common basic dyes may have different colors from their salts, and that they are soluble in neutral fat, xylol and alcohol, but slightly soluble or insoluble in water. It was also shown that the hydrolysis of neutral fat can be demonstrated by dissolving a suitable dye base in the neutral fat and observing for the shift in the color of the solution toward that of the salt. It has also been shown (4) that the degree of development of color of the soap is directly related to the concentration of the free fatty acids, and that fact formed the basis of a quick test of the quality of butter. In the present paper, we are concerned with the application of the above principle in a general procedure for the determination of free fatty acids in milk fat, a procedure which, obviously, can also be used for the same purpose in the study of fat in other dairy products.

PROCEDURE

The sample of milk fat was prepared from milk by the method of Herrington and Krukovsky (1) and the concentration of fatty acids was determined both by titration with 0.05 N NaOH (Herrington and Krukovsky (1)) and by the present procedure which is similar to that used by Knaysi and Guthrie (4) modified to fit the requirement of exact analytical work.

One ml. of the sample of milk fat was measured into a test tube which had been painstakingly cleaned with dichromate cleaning mixture, liberally rinsed with running and distilled water and allowed to dry in the oven. To this ml. of milk fat 3 ml. of a saturated solution of neutral red base in chemically pure xylol were added and the tube was gently shaken to promote dissolving of the fat. The color of the solution is then compared to that of standards containing known quantities of oleic acid in test tubes of approximately the same diameter. That can be done immediately or at one's leisure, for when the tube containing the milk fat is tightly stoppered with a cork lined with a piece of tin foil, no change in its color is detected even after standing for 2 days. Corks that have been exposed to acid or alkaline fumes and vapors should never be used in this work.

The standards are prepared as follows: 0.5647 gm. of pure oleic acid is weighed in a 50 ml. volumetric flask, and the flask is filled to the mark with chemically pure xylol; 1 ml. of this solution is also diluted with xylol to 10 ml. That gives, respectively, a 0.04 and a 0.004 normal oleic acid in

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xylol. Now ten test tubes cleaned and dried as mentioned above, and of the same internal diameter, are labelled 0, 1, 2, 3, 5, 7, 10, 12, 15, 20. To each test tube is added oleic acid solution, pure xylol and saturated neutral red base solution in xylol in the quantities indicated in table 1. It is obvious that when the standards are thus prepared and labelled, the number of the standard gives the normality of oleic acid multiplied by 10^{-4}. The standard containing no oleic acid is orange yellow with a green fluorescence, while the other standards have a reddish tinge in proportion to their oleic acid content. They are stable and can be kept for weeks when tightly stoppered with corks free from acid or alkali vapors and lined with tin foil. When not in use, the standards should be kept in a cool, dark place. The above series is considered adequate for it is possible to estimate shades intermediate between those of two consecutive standards.

TABLE 1

Preparation of the standards

No. of standard	Ml. of oleic acid		Ml. of xylol	Ml. of satur. dye base sol.	Normality of oleic acid
	0.04 N	0.004 N			
0	0.00	0.00	1.00	3.0	0.0000
1	0.10	0.00	0.90	3.0	0.0001
2	0.20	0.00	0.80	3.0	0.0002
3	0.30	0.00	0.70	3.0	0.0003
5	0.50	0.00	0.50	3.0	0.0005
7	0.70	0.00	0.30	3.0	0.0007
10	1.00	0.00	0.00	3.0	0.0010
12	0.00	0.12	0.88	3.0	0.0012
15	0.00	0.15	0.85	3.0	0.0015
20	0.00	0.20	0.80	3.0	0.0020

DATA AND DISCUSSION

A comparison between the results of titrations by the procedure of Herrington and Krukovsky (1), expressed as normality of fatty acids, and those determined by the present method are given in table 2 and show close agreement. The 18 samples of milk fat originated from six different lots of milk. Each lot was divided into 3 portions, the first portion was pasteurized immediately and the two others cooled at different rates. The data, therefore, confirm the finding of Herrington and Krukovsky (2) that a slow rate of cooling of the raw milk promotes lipolysis.

In view of the very low concentration of free fatty acids in normal milk, and the fact that only 1 ml. of the milk fat is required, it may be concluded that the method we are presenting is of very high sensitivity. Differences in the carotenoid content of various milk, in the dilution used and in the presence of the base of neutral red, seem to have no noticeable effect on the accuracy of the method, and the color, which is due to intramolecular rearrangement of the base upon salt formation, is independent of the nature of

TABLE 2

The free fatty acid content of milk fat

Sample No.	Normality of free fatty acids		Remarks
	Dye base method	Titration with NaOH	
1	0.0005	0.0005	Immediately pasteur. Control
8	0.0026		Cooled in can*
9	0.0008	0.0009	Cooled over surface cooler
2	0.0007	0.0008	Immediately pasteur. Control
10	0.0010	0.0012	Cooled in can
11	0.0007	0.0008	Cooled over surface cooler
3	0.0006	0.0006	Immediately pasteur. Control
12	0.0019	0.0019	Cooled in can
13	0.0008	0.0010	Cooled over surface cooler
4	0.0005	0.0005	Immediately pasteur. Control
14	0.0011	0.0013	Cooled in can
15	0.0006	0.0007	Cooled over surface cooler
5	0.0005	0.0005	Immediately pasteur. Control
16		0.0019	Cooled in can
17	0.0008	0.0009	Colled over surface cooler
6	0.0006	0.0006	Immediately pasteur. Control
18	0.0011	0.0013	Cooled in can
19	0.0007	0.0008	Cooled over surface cooler

* 40 qt. can.

the fatty acid and is the same for the same normal concentration of the various soaps formed.

SUMMARY

A simple and quick colorimetric method for the determination of free fatty acids in milk fat is described. The method consists in dissolving 1 ml. of the milk fat in 3 ml. of a saturated solution of the base of neutral red in xylol and comparing with a set of standards of known oleic acid contents. The method is shown to be highly sensitive and accurate. In neutral fat and in xylol the dye base gives an orange yellow solution. Free fatty acids form red soaps with the dye base and the degree of shift to the red is proportional to the concentration of soap and, therefore, of the free fatty acids. Equal normal concentrations of various fatty acids produce an equal shift in the color.

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A METHOD FOR SURFACE AREA MEASUREMENT OF MILK BOTTLES*

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The problem of the horticulturist in calculating the leaf surface of a fruit tree for measurement of the spray covering is similar to that of the milk plant manager or experimentalist who wishes to determine the amount of water and washing powder required to wash milk bottles of varying sizes. Many factors complicate his problem, chief of which is the fact that usually three sizes of bottles are washed, the total number varying each day, as well as the distribution of sizes. This serves to make any calculation of washing powder only approximate. Other factors, also confusing, are shape of the bottle and thickness of its walls.

Displacement calculations may be utilized as a simple method for determination of the volume of glass in a bottle but they are valueless as a measure of the surface area, due to irregularity in shape. Since both the inside and outside areas of the bottle must be washed it is necessary that both be measured. From these values may be obtained a ratio from which the areas of pint and half-pint bottles may be converted into quart-equivalent area. It would seem logical to calculate amounts of water and washing powder required in washing bottles in terms of the amounts required to wash a quart bottle. In the discussion which follows, the surface areas of three standard size milk bottles have been calculated and the method of calculation given.

The approximate area of the outer and inner surface of a milk bottle can be found by dividing the bottle into small sections as is shown in fig. 1. Each of the sections is a frustum of a cone whose surface area is:

$$\text{Area} = \frac{\pi}{2} s[(D_1 + D_2) + (d_1 + d_2)],$$

where s is the slant height, D_1 and D_2 are respectively the upper and lower diameters and d_1 and d_2 are respectively the upper and lower inside diameters. In figure 1 (a) is shown one of these sections in detail; this is section SRUT which is located on the neck of the bottle and is similar to the other sections. The various diameters D_1, D_2, d_1 and d_2 together with the slant heights are shown. The sum of the outer and inner surface areas of this small section is:

$$\text{Area} = \frac{\pi}{2}(RT)[(RS + TU) + (R'S' + T'U')]$$

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The sections were taken so that the slant height fell along a straight edge. The slant height of the above section is RT and it is a straight line. Where the curvature of the surface of the bottle was large, heights of the sections were small, some of them being as small as 1/32 inch. At places where the curvature was large there were several sections. Section KMBA was the largest, for on this part of the bottle the lines MB and KA were straight lines. There were eleven sections between KM and TU, four at the bottom of the bottle and six at the top. It was possible to divide the bottle into frustums of cones by making the sections small enough.

The diameters RS, KA, CD, etc., were measured with a caliper which read to hundredths of an inch. After the bottle was broken in several places thicknesses of the glass were measured. The inside diameters were obtained by subtracting twice the thickness of the glass from the corresponding outside diameters. The thickness of the glass varied for different parts of the bottle. The neck consisted of thicker glass than the body of the bottle, the glass at the top being the thickest. The bottom of the bottle on the inside was shaped like a cone. Its area was found by using the formula for the surface area of a cone. The part of the bottom which rested on a flat surface was a circle. The very top of the bottle was a ring as is shown in figure 1(b). The area of this ring was found by subtracting the area of the inner circle from the area of the outer circle. The following table contains the surface areas of a quart, a pint, and a half-pint milk bottle.

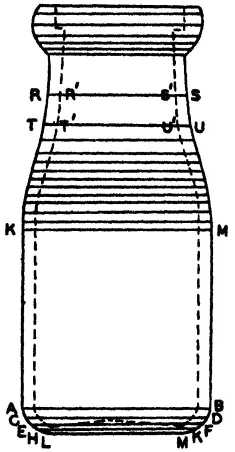

Fig. 1.
Half Pint

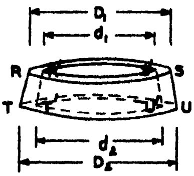

Fig. 1a.
Section RSUT

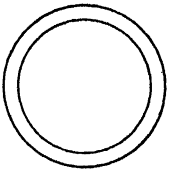

Fig. 1b.
Top Ring

The ratio of the surface area of the pint and the surface area of the quart is 0.62; the ratio of the area of the half-pint and the area of the pint is 0.66. The ratio of the area of the half-pint and the area of the quart is

Surface area of milk bottles (sq. in.)

Surface	Quart	Pint	Half-pint
Outside	106.58	65.64	43.52
Inside	93.82	58.24	38.81
Outer and inner surfaces	200.40	123.88	82.33

0.41. Small errors may have been made in measuring the various parts of the bottles. The above figures can be taken as very close approximate values of the surface areas of these three milk containers.

The surface area of a quart bottle of the design used in these calculations is 1.62 times greater than that of a pint bottle and 2.43 times greater than that of a half-pint bottle. To convert pints and half-pints to their quart equivalents merely multiply the numbers by their respective ratio. Thus, if 1000 quarts, 200 pints, and 800 half-pints were to be washed, the quart equivalent would amount to 1000, plus 200 × 0.62, plus 800 × 0.41, or an equivalent of 1452 quarts.

ABSTRACTS OF PAPERS PRESENTED AT THE THIRTY-SEVENTH ANNUAL MEETING

June 22–25, 1942

EXTENSION SECTION

Testing Committee Report

A. Maintaining Qualified Tester Personnel. A. J. Cramer, Univ. Wis., Madison, Wis.

"Greater Production for Defense and Victory," depends on maintaining qualified Supervisor Personnel.

A. Locating and training Prospective Supervisors.

1. Streamlined Supervisor Courses.

A five-week supervisor or fieldman's training course is given each year in February and March to boys attending the Short Course at the Wis. College of Agriculture. In addition to other classes, a two-hour-a-day class was taught by the staff of the Dairy Records Office. Studies on Dairy Cattle Breeding, the application of records and the duties of fieldmen were emphasized. The course involves the testing of milk, balancing of grain rations, the filling out of monthly reports and other forms. Records for a barn book and a sample members herd record book are kept by the students on a herd of a member for one complete years work. Instructions are given on services a member should receive, methods of organizing and maintaining a Standard Dairy Herd Improvement Association.

Many of our Smith Hughes High School Agricultural teachers are offering courses to students as preliminary training for a supervisors job.

One- or two-day training courses, under the supervision of the Dairy Records Office, have been offered to prospective fieldmen at High Schools throughout the state.

Our standard D.H.I.A. fieldmen have trained applicants by allowing these men to do the Supervisors work on a number of farms which work is supervised by the regular fieldman. This method has proved most satisfactory when the better trained supervisors give the instructions.

We were only fairly successful in locating prospective supervisors by placing a "Help Wanted" ad in a State Farm Paper, which read: "Dairy Fieldman for Testing Associations—High School Agricultural Course or Short Course graduates preferred." County Agents have been fairly successful in locating boys on farms within the county, through advertising in local papers.

B. Deferment of Supervisors.

Our County Agents, D.H.I.A. Members and Members of the Dairy

Record Office have been fairly successful in retaining Supervisors by writing letters to the local draft boards, explaining the importance of deferring the Supervisors. In many instances the draft boards have deferred the men for a third time.

C. Maintaining Equipment of Supervisors.

1. Cars and Tires.

A letter was mailed to all County Agents and D.H.I.A. fieldmen, the contents of which was taken from a letter written by J. F. Kendrick, Chief, Division of Dairy Herd Improvement Investigations, Bureau of Dairy Industry. Here it is: "D.H.I.A. Supervisors may obtain recaps under the rating B–A–3. This rating includes passenger cars used principally for providing transportation to technicians, essential to the war effort in the field of Agriculture." The D.H.I.A. program is regarded as essential to the *More Milk* Program.

One of our fieldmen obtained a new car soon after the rationing board received the request. Some boards refused to grant recapped tires to fieldmen, because the board had not received the priority rating literature. Their cases are pending.

2. Glassware and Acid.

Sulphuric Acid and Glassware have been obtained so far, since the Office of Production Management submitted the following information Preference Rating Order No. P–62, which deals with materials for the production of laboratory equipment and reagent chemicals. This order should make available without special preference ratings, all the reagent sulphuric acid that might be needed for milk testing.

D. Results.

So far we have been able to maintain a large number of our Associations with Supervisors by advertising for help wanted and by giving courses helpful toward the training of new men. Some draft boards have been lenient with our supervisors by deferring them.

Our fieldmen have been fairly successful in getting equipment as glassware and acid and also in obtaining used and recapped tires.

B. Supervisory Problems. H. E. Loveland, Univ. Vt., Burlington, Vt.

The war situation is making it necessary to adjust D.H.I. association work so it will reach the largest number of producing cows with a shortage of testers, and testers with limited training, experience, and qualifications for the job. These conditions make it necessary for the membership to realize the need of adjustments and to be willing to cooperate in the use of methods which are new and often not popular. To secure these adjustments without loss of members, and with minimum loss of accuracy requires:

(1) that associations be locally organized with active management by directors, and

(2) that membership be kept informed through letters and occasional meetings.

Probably most associations in the country are so organized, but often the officers do not feel their responsibility. Monthly letters from the state office, annual reports, occasional letters for special information, annual meetings, conferences of groups of officers, are methods which may be used.

The high value of D.H.I. association testing for promoting the increased production goals set by the U. S. Department of Agriculture requires the coordination and cooperation of different associations in a territory to keep the records coming. It is better to have acceptable records from all herds than good records from a few herds, and none from the balance. Thus, the supervision should include furnishing information on how goals are being reached, methods of reaching same, the necessity of continuing records, and how associations can cooperate with each other.

The above statement does not mean that we should let down on the aim of high standards and we should educate the testers and help in selection of testers to do the best job possible. When these standards cannot be fully maintained, we should aim for as many records as possible on an acceptable basis for publicity and proving of sires.

The extension of a testing program to other dairymen should be on basis of publicity of sources of D.H.I. association tested herd sires, methods of herd management successfully used by D.H.I. association members, and adoption of other forms of keeping records which will give those unable to join a D.H.I. association some kind of private records for use in herd improvement.

C. Emergency Adjustment in D.H.I.A. Procedure. C. R. GEARHART, Pennsylvania State College, State College, Pa.

War conditions make it necessary to make adjustments in our D.H.I.A. procedure. Problems confronting Supervisors are numerous, including: How to keep the D.H.I.A. program up to standard with a shortage of testers, tires, gas, paper, and proper supervision.

This paper shall attempt to outline Emergency Adjustments made by the various states up to April 1, 1942, and to list suggestions for further adjustments.

Special attention will be given to:

1. *Cooperative Grouping of D.H.I.A.'s.*

Under this system a number of associations agree to exchange testers, temporarily, in order that no member shall lose more than one month's testing at a time. With 8 associations in the group it would mean in a case of a shortage of testers, all members may miss one or two month's testing during the year, but none will lose out entirely.

2. *Multiple Testing.*

a. Doubling herds.

The regular method of doubling herds as mentioned in the Tester's manual.

b. Doubling testers or Tester and Assistant.

This will include two testers using one car to save tires, or a regular tester taking an assistant with him, in some cases it may be his wife. The assistant will be dropped at one farm while the regular tester goes to the next farm, the two getting together to do the testing and record work. This gives the regular tester an opportunity to supervise the work of his assistant.

3. *Laboratory Testing* (*disinterested sampler*).

Under "Laboratory Testing" is the method of having samples taken by disinterested samplers and brought in to a regular tester, or to a central laboratory for testing. These samplers may be a group of regular testers, or they may be 4–H club members, Vo–Ag students, or local dairymen who take samples on other than their own herds.

4. *Bi-Monthly testing* is the regular method as outlined in the Tester's Manual.

Due to unforseen circumstances, it will undoubtedly be necessary to make additional adjustments from time to time.

D. Current Developments Affecting D.H.I.A. Work. JOSEPH B. PARKER, Senior Extension Dairyman, U.S.D.A.

The major problem facing the D.H.I.A. program has been that of locating and training a sufficient number of replacement testers. Not only has there been no decrease in interest on the part of dairymen for the testing work, but a considerable number of new associations would have been organized if testers had been available.

Local selective service boards have been very cooperative in most cases and many testers have been deferred. In spite of this, a considerable number of testers either have volunteered for the armed services or have taken better paying jobs in defense plants. The turnover in the tester personnel has been terrific. The alternatives facing the state dairy specialists have been women testers, men over service age or with some physical handicap that would make them ineligible for military duty, boys of 18 or 19 years of age, and bi-monthly testing. All of these methods are being tested in various associations at the present time.

Another problem facing the work has been tires for testers. This has caused considerable uncertainty on the part of testers whose tires were getting smooth. A number of tire-rationing boards have now certified testers for tire recapping so that this picture is a little brighter.

The next problem to be faced by the testers will be under the gasoline rationing program. So far no reports have been received from the rationing states that testers have been unable to obtain "X" cards. If gas rationing is extended to the whole country after July 1, as reports now indicate, an attempt will be made to obtain a ruling on the eligibility of testers for the required amounts of gas to cover their regular travel.

Sire Committee Report

B. Present-day Techniques of Artificial Insemination. GEORGE W. TRIMBERGER, Univ. Nebr., Lincoln, Nebr.

Information for this discussion on present-day techniques of artificial insemination was obtained from questionnaires sent to 64 artificial breeding associations located in 17 states. There was quite a variation in the essential techniques followed, but a study of the reports at hand indicated that the best results are obtained by using the technique described below. This is confirmed by experiments with artificial insemination at the University of Nebraska dating from 1936 to the present.

The hot water used in the artificial vagina ranges in temperature from 110 to 140° F., depending upon the individuality of the bull and the season of the year. Increasing the temperature and pressure in the artificial vagina and inserting the penis into the vagina immediately when the bull starts mounting have been found helpful if a bull fails to ejaculate. The frequency of use for the bulls is an important factor for successful operation of a breeding association and may have an influence on the breeding record as well as on the time of successful storage for the semen from a bull. Two ejaculations taken every third or fourth day with an occasional rest from sexual activity usually produces best results.

Data from the breeding associations in different states indicate little variation in the procedure followed for diluting and storing semen. Two diluters are extensively used with about equally good results. One of these, known as Phillips or Wisconsin Egg Yolk Phosphate Buffer, is described in the *Journal of Biological Chemistry,* 130: 145, 1939; and the other, which is a yolk citrate buffer containing equal parts of fresh egg yolk and a M/15 solution of sodium citrate, was suggested by Salisbury, Fuller, and Willett in the JOURNAL OF DAIRY SCIENCE, 24: 905, 1941. It is important that this egg yolk diluter be prepared as needed from fresh eggs. The eggs used in the diluter should be refrigerated soon after they are laid because if this is not done, it is believed, certain changes take place in the eggs within a few hours and these changes may be detrimental to the sperm cell. The diluter is added to the semen as soon after it is taken as is possible but not until both are of the same temperature. The ratio of dilution often is 1 part of semen to 3 parts diluter but other dilutions are used. If any diluter is used to rinse the vagina at the time of collection, it should be the same as is later used for dilution of the semen. There is a general agreement that semen should be cooled gradually but that no definite procedure is necessary to warm it before use. Various means are used to cool the samples gradually. Some technicians use double-walled test tubes; others wrap the tubes in flannel or other material; and still others prefer placing the sample tube in a jar of water large enough so that cooling takes place at the rate of 1° F. per minute when the sample is placed in the refrigerator. About 40° F. is the common storage temperature.

The preferred method for inseminating females is to use a surgical rubber glove and sleeve and hold the cervix through the intestinal wall so that the inseminating tube can be guided through it in the majority of cases. The use of a speculum often does not permit the inseminating tube to be inserted through the cervix. Experiments at Nebraska have indicated that it is best to breed in the middle or toward the end of estrus, although good results are obtained from services as late as six hours after the end of estrus, but beyond this point poor results are obtained. About 1 to 1.5 cc. of the diluted semen is usually considered a sufficient quantity for each insemination. When a 3 to 1 dilution is used, the females actually receive .25 to .4 cc. of semen. At the present time, many artifical breeding associations successfully use semen (with 50 per cent or more conceptions) that has been stored for a time interval up to 72 hours. Several subsidiary breeding associations in New York do not receive any semen that has been in storage less than 24 hours.

The keeping of proper records so that the breeding history of each service bull and female can be properly studied is important. If a bull drops below 50 per cent conceptions in the females to which he is bred, he should be withdrawn from service or used only to a limited extent. The semen from some bulls can be successfully stored for a much longer time than that from others, and the records should reveal this so that the samples can be used accordingly. It is recommended that the females be eartagged for easy and proper identification. When a female has had three services without conception, it is not advisable to breed her again until she has been given a thorough examination and the trouble diagnosed.

If the percentage of conception is not satisfactory, some change should be made to rectify the condition. Lack of sanitation and improper sterilization of instruments have been found to result in poor breeding records in several associations. Bulls with a high breeding efficiency can be selected for service, and sometimes the use of younger bulls can be justified on this basis. Storage conditions of the semen and the inseminating technique can often be improved. The health and general condition of the herds should be carefully observed. Cows in herds with Bang's disease have often been found very difficult to settle. An attempt should be made to increase the dairymen's knowledge of the phases of reproduction. Dairymen should understand, too, that a small percentage of females are non-breeders and have to be sold as sterile. Furthermore, a considerable number of females requires several services for conception. Any figure over [illegible]0 per cent of conception in the females bred or a requirement of less than 2 services per conception can be considered a satisfactory breeding record.

C. Storing, Packaging and Shipping Semen. G. W. SALISBURY, Dept. Animal Husbandry, Cornell Univ., Ithaca, N. Y.

Eighteen of the twenty-two artificial breeding cooperatives in New York are member units of one large organization, the Central New York Artificial Breeders' Cooperative. The bulls which supply semen to the member units are all housed near Syracuse, and from that point the semen is shipped to the local inseminators of each member unit. In some cases the semen is shipped 200 miles or more, and the ejaculate collected on one morning may not reach its destination until the next day.

Semen, as handled when artificial insemination was first used in New York, could not be depended upon to maintain its fertility for more than twenty-four hours after collection. Today as a routine practice the semen is not only shipped many miles, but is often used for at least four days after collection and occasionally much longer. This change has been brought about by many factors, among which may be mentioned the use of appropriate diluters, proper cooling of the semen, development of satisfactory shipping containers so as to control the temperature of semen in transit, and development of time-saving devices which enable the operator of the laboratory to rapidly control to a large extent the quality of the semen which is shipped.

The paper which is to be presented will briefly set forth the results of four years of intensive research on certain of these problems. The application of the research findings to the solving of practical problems which arise when the semen must be assayed as to quality, properly prepared for storage, satisfactorily packaged and shipped to a relatively distant point before use, will be discussed.

Feeding Committee Report

A. Simple vs. Complex Rations for Dairy Cattle. C. F. MONROE AND W. E. KRAUSS, Dairy Dept., Ohio Expt. Sta., Wooster, Ohio.

In Ohio as in other corn belt states, corn is generally the cheapest grain for the dairy ration. Hence a simple grain mixture composed of corn and soybean oil meal, which in the past has been comparatively low in price, together with the necessary salt and minerals, offers possibilities for reducing feeding costs. From an experimental point of view, a simple grain mixture also offers possibilities for obtaining information on the value of feeds which may be added to or substituted in the mixture.

The work to be presented should be considered as a progress report rather than the final answer. In a reversal trial, twenty-eight Holstein cows, divided into two groups, received in alternate periods of 50 days each, simple and complex grain mixtures. The simple grain mixture was made up as outlined previously and the complex mixture contained in addition to these ingredients, oats, linseed oil meal, wheat bran, beet pulp and molasses. This complex mixture had been in use previously and was considered highly satisfactory, cost excepted. These mixtures were fairly comparable in respect to digestible protein and total digestible nutrient content. Along with these grain mixtures, all the cows received a moderate amount of corn silage and liberal amounts of legume hay (fed ad lib.). The cows were milked and fed three times a day. The production level per cow was around 1200 pounds of milk and 37 pounds of butterfat per month. For this short-time trial there was practically no difference in the results from the two grain mixtures.

In a further comparison of the simple and complex mixtures other groups of Holstein cows were fed the same mixtures while on pasture for a period of 120 days. In this work the groups were not reversed but were continued on their respective rations for the full pasture season. Because the pasture consisted chiefly of legumes and hence furnished liberal amounts of protein, two other simple grain mixtures of lower protein content were fed to still other groups of cows. This pasture trial confirmed in general the results of the barn feeding trial.

As a side issue to this work, the question has arisen of whether to use ground shelled corn or ground ear corn in the simple mixture. During this last winter two feeding trials have been conducted to answer this question. The data for these trials in which a large number of cows have been used will be presented.

The comparison of simple and complex grain mixtures has been extended to calf feeding. In the calf rations the simple grain mixtures contained oats in addition to corn but no soybean oil meal. The data to be presented are based on the results obtained with 65 heifer calves from birth to 6 months of age and on 58 bull calves from birth to 9 weeks. The results with the younger animals also indicate the possibility of the simple ration.

Quality and Marketing Committee Report

B. Organization of a Quality Improvement Project. C. J. BABCOCK, Bureau of Dairying, U.S.D.A., Washington, D. C.

A quality improvement project for milk and cream will best accomplish the desired results if it is organized on an area plan. The area should comprise all the producers supplying an entire buying area. This plan is an indirect method of aiding the producer to improve the quality of milk and cream. Experience has shown that working directly with producers not only fails to accomplish the desired results but it is costly and time-consuming. As extension dairymen you are too busy to even attempt to contact all milk producers in your territories. I believe, however, that most of you do have the time to assume the leadership and direction of an area project. The cooperators in such a project have been outlined by your committee chairman. Obtaining the cooperation of these parties is mainly a matter of leadership. For the most part they are familiar with the necessity for improving quality. They have realized that something should be done, but have left it to the other fellow. In the past the cooperation of the buyers of milk and cream has been the hardest to obtain. Their cooperation is essential. They are responsible for the quality of our dairy products. They are solely responsible for the quality of the milk and cream which enter into manufactured products. They have, however, often placed volume of dairy products above quality of dairy products. They have frequently judged their success by the volume handled. The result has been that they have accepted milk and cream which they knew was of a quality unfit for use. They accepted it because they knew that a competitor would accept it in order to increase his volume. There never was a more opportune time to obtain the cooperation of the buyers of milk and cream than the present. The increased demand for dairy products, which our producers are meeting with increased production, has caused a large percentage of our plants to operate to capacity. In other words, their volume complex has been, at least temporarily, satisfied. While operating to capacity is the time when they more readily respond to plans for preventing the entrance of unfit milk and cream into the channels of human consumption.

The first step in organizing a quality improvement program should be a definite outline as to what you expect to do and how you expect to do it. This outline should be discussed with the cooperating agencies and have their approval. The next step is to obtain the cooperation of all those buying milk and cream in the area chosen. Personal visits should be made to the buyers in order to arouse interest prior to calling a meeting of these men at which the project will be explained in detail. The buyers must agree to make certain quality tests on all incoming products and reject all milk and cream that does not meet an acceptable standard. Control officials should make frequent checks to determine that the quality tests are similarly conducted and the results similarly interpreted at all the plants. They should also determine that all milk and cream not meeting the acceptable standard is actually rejected. The dairy manufacturing section of the college should cooperate in determining what quality tests are used and at what point, according to the tests, the milk and cream are to be rejected. They should also cooperate when necessary by instructing plant operators how to conduct and interpret quality tests.

As soon as the program has been definitely planned and all cooperating agencies contacted the extension service should hold meetings of all the patrons selling milk or cream in the area. These meetings can probably best be called by the dealers. At these meetings the extension service completely outlines the program. The producers are informed as to its objects and benefits. Demonstrations of the quality tests are made so as to show the difference between good and poor quality and what milk or cream will be rejected. It is explained to the producers that the results of the quality tests made at the plants are given to both the extension service and to the control officials in order that these agencies may know where best to concentrate their efforts in improving quality. It is also explained that the extension service and control agencies are going to check the buyers on the conduct of the tests to see that all producers receive equal treatment and that poor-quality milk and cream is actually rejected.

The better producers in the area should be appointed as key men to aid and advise their fellow dairymen and to take an active part in the program. These better dairymen realize the importance of quality and can be of great service to the extension service in explaining the progress of the program at meetings of producers, which should be held at frequent intervals. The appointing of key men to take an active part in the program is the best possible means of getting the producers to realize that this is their program, for their own benefit, and not something that is being forced upon them.

An educational program is continuously carried on among the producers. The extension service furnishes the material; bulletins, films, etc. Bulletins should be furnished to the producers through the county agents, the dealers, or both. Demonstrations, talks, and films on quality improvement should be left largely to 4-H dairy clubs and vocational high school students. Quality improvement of milk and cream is an ideal project for 4-H clubs. They not only make an ideal medium with which to get the message across to producers, but they are an ideal training for future dairymen.

The extension service should make full use of all the cooperating agencies as listed by your chairman. Many of these agencies have men in the field who can do promotional work for the project and assist producers with their problems. Furthermore, the cooperation of local newspapers, etc., should be obtained in order that the program may get full publicity. This publicity should start with the organization of the project, at which time the object and methods are published in detail. After the project is organized the publicity may be in the form of progress reports. Newspaper publicity not only adds impetus to the program, but it is an ideal medium for keeping those interested informed as to the progress of the program and the accomplishments.

To briefly summarize, the main duties of a dairy specialist in quality improvement work on the area plan are organization and direction. The actual producer contact is made by his cooperators and the condemnation and rejection of milk and cream are made under the direction of control officials. Demonstrations and presentation of films, etc., are carried on by 4-H clubs and vocational agriculture schools. The extension service localizes its assistance to those actually in need of assistance as shown by quality tests and this work is largely done by the county agents. It is an opportunity to carry on a quality improvement project with less expenditure of time and effort than the average dairy specialist spends in talking quality and giving demonstrations at producer meetings. It is a project that actually shows results which can be definitely measured. During the present emergency this program should deal mainly with milk and cream for manufacturing purposes. In many localities it could be further limited to milk which is to be manufactured into lend-lease products.

C. Dairy Manufacturing Activities Devoted to National Defense. J. M. Jensen, Extension Dairy Specialist, Michigan State College, East Lansing, Mich.

Demands on the Dairy Industry for large quantities of cheese, evaporated milk and skim milk powder for defense needs created many problems that required the assistance of specialists in Dairy Manufacturing. These problems dealt with surveys of factory output and capacity, promoting diversion from surplus areas to needed factories, determining best solution to problems arising for different localities, assisting with estimates on equipment layout and cost, pointing out the added need for quality and assisting in formulating quality programs and factory operations to that end.

Further defense activities required programs for improving milk and milk products of all classes, as a means toward furthering consumption, improving keeping properties and reducing losses.

PRODUCTION SECTION

P1. Improving Dairy-Cattle Pastures. W. B. Nevens, Univ. Ill., Urbana, Ill.

Alfalfa, bluegrass, brome grass, winter rye, sweet clover, and a mixture of Sudan grass and soybeans grown in separate fields were combined in a rotational pasture system. Winter rye furnished an abundance of early spring pasture. The rye field was seeded in late May to Sudan grass and soybeans. The cattle were transferred from one crop to another as soon as the best part had been eaten and before the plants became too short to make a vigorous new growth.

The outstanding results of seven years' trials were: (a) The pasture season was extended from two to six weeks longer than the period of pasturing bluegrass; (b) The yields (dry-matter basis) were from two to three times as great as those from improved bluegrass pastures used alone; (c) A good supply of green, palatable feed was maintained throughout the season; (d) The weed content of most of the pastures was much less than that of the bluegrass pastures; (e) The most important factor in improvement of yields was the selection of suitable crops and the combining of these in a rotational grazing system; (f) The second most important improvement factor was the heavy application of barnyard manure; (g) The application of cows' urine increased the protein content of bluegrass from 10 per cent to 25 per cent above that of bluegrass treated with barnyard manure; (h) The urine-treated bluegrass was more palatable than the untreated. This was found by a statistical analysis of 318 dry-matter determinations of treated and untreated bluegrass to be explained by the lower dry-matter content of the treated grass; (i) Probably the most important factor determining the palatability of dairy-cattle pasture crops is their dry-matter content. The crop lowest in dry-matter content, based on all samples taken during May to September, inclusive, over a 5-year period, was Sudan grass-soybeans. This was followed in order by winter rye, alfalfa, brome grass, sweet clover, and bluegrass. Of 389 bluegrass pasture samples, 92 per cent contained more than 30 per cent dry matter, while only one of 38 Sudan grass-soybean pasture samples was higher than 30 per cent in dry matter content.

P2. The Ability of Yearling Heifers to Withstand Cold Temperatures.

J. R. DICE, Dept. Dairy Husbandry, North Dakota Agricultural College, Fargo, N. Dak.

Work done at this station which we have previously reported[1] indicated that: (a) dairy cows can withstand exposure to cold temperature; (b) that they will produce practically the same in a cold stable as they will in a stable where the temperature is about 50° F.; (c) that milk cows on full feed, when housed in a cold stable produce sufficient surplus heat over usual maintenance requirements to maintain body temperatures without using nutrients for that purpose; (d) that cows housed in a cold shed require no more protein and total digestible nutrients for milk and butterfat production than other cows or the same cows when kept in a standard dairy barn; (e) that the cows in the cold shed tend to gain somewhat more body weight than the cows in the dairy barn.

We have housed yearling heifers in an open shed where the average temperatures were below freezing and another group in a closed shed where the temperatures were usually above freezing. The data accumulated to date indicate that the heifers in the closed shed average to put on more weight, and grow more as indicated by the gain in height at withers than the heifers in the open shed. Results obtained during two seasons confirm definitely the above statement. Results obtained during the third year do not. Averages indicate that the heifers in the closed shed made the best gains in skeleton growth in each of the three years and the best gains in weight during two of the three years.

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[1] Dice, J. R. The Influence of Stable Temperature on the Production and Feed Requirements of Dairy Cows. JOUR. DAIRY SCI., 23, No. 1: 61–69. 1940.

This is to be considered as a progress report as the project is to be carried on at least through two more seasons.

P3. Resting Maintenance Cost in Growing Dairy Cattle.[*] S. BRODY, H. H. KIBLER, AND A. C. RAGSDALE, Dept. Dairy Husbandry, Univ. Mo., Columbia, Mo.

The manner in which resting maintenance cost (resting energy metabolism measured by rate of O_2 consumption) of Jersey and Holstein cattle varies with increasing age and body weight is graphically illustrated. Prior to about five months, the metabolism tends to vary directly with body weight (about 24 cal./pound/day); following this age, the metabolism varies not with $W^{1.0}$, but with $W^{0.6}$, meaning that increasing body weight one per cent increases maintenance cost only about 0.6%. From the surface-area viewpoint, the metabolism per square meter rises from birth until age of "natural weaning" (5 to 6 months) and remains approximately constant thereafter.

P4. The Occurrence and Importance of Still-unidentified Nutrients in Milk and Milk Products. A. M. HARTMAN AND C. A. CARY, Division of Nutrition and Physiology, Bureau of Dairy Industry, U.S.D.A., Washington, D. C.

Young rats nursed by mothers fed a diet of hot-alcohol-extracted casein, dextrin, yeast, cottonseed oil, cod-liver oil, agar, and a salt mixture did not grow normally when continued, after weaning, on this diet or on this diet containing additional yeast (bakers' or brewers') or supplemented with an average daily dose of thiamin chloride 40 μg., riboflavin 40 μg. or 160 μg., pyridoxine hydrochloride 40 μg., nicotinic acid 0.5 mg., calcium pantothenate 0.5 mg., inositol 0.5 mg., para-aminobenzoic acid 3.0 mg., choline chloride 6.0 mg., or biotin methyl ester 1.0 μg., or by vitamins E (alpha-tocopherol) 50 μg. or K (2-methyl, 1,4,-napthoquinone) 12.5 μg.

The rate of growth may be increased 90 per cent or more by replacing the extracted casein with commercial casein or by feeding various liver extracts separately from the basal diet. Feeding a liver fraction which furnished only 0.2 mg. of solids daily produced decided growth. In paired feeding experiments (still in progress), males which have been fed, separately from the basal ration, a quantity of liver extract that furnished an insignificant amount of metabolizable energy, have grown more than sex-litter mates on the basal ration alone. The average difference is not large

[*] Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 833.

but it is statistically significant. Accompanying the increased rate of body growth of females fed liver extracts were earlier vaginal patency and marked increases in the weights of the ovaries, the uterus and the adrenals; similarly with males, marked increases were observed in the weights of the seminal vesicles and prostate, the epididymis and the thymus.

Practically colorless, active liver extracts have been prepared; synthetic xanthopterin was inactive; and the growth-promoting material in the liver preparations was not precipitated by ammoniacal silver nitrate as is xanthopterin. The factor in liver extracts resembles pyridoxine in some of its chemical properties. Aqueous solutions of a liver extract, adjusted to pH's 3, 7, and 11 and then autoclaved at 18 pounds pressure for 3 hours, remained active but possibly suffered some loss in activity in each case. Fresh whole milk, cheeses (cottage, Swiss, Cheddar), beef muscle, and lettuce promoted growth when fed in addition to the above basal diet; commercial dried skim milks had a similar effect.

P5. Hydroxyamino Acids of Milk Proteins.

B. H. NICOLET, L. A. SHINN, AND L. J. SAIDEL, Division of Nutrition and Physiology, Bureau of Dairy Industry, U.S.D.A., Washington, D. C.

The discovery that periodic acid reacts with amino alcohols has led to the development of satisfactory methods for the determination of serine and threonine, and also to a demonstration that hydroxy glutamic acid probably does not occur in milk proteins.

The use of these methods has made it possible to show that serine and threonine, in protein combination, have a special sensitivity to alkali analogous to (but less than) that of cystine. Seryl residues, for instance, are dehydrated to give dehydroalanyl residues, the structure of which is clearly shown by their ability to add substances of the type RSH to form cysteyl derivatives.

When a protein containing such residues is subsequently submitted to acid hydrolysis the nitrogen in them comes off as ammonia.

P6. Utilization of Urea by Calves Less than Four Months of Age.

J. K. LOOSLI, C. M. MCCAY, AND L. A. MAYNARD, Cornell Univ., Ithaca, N. Y.

Holstein calves receiving whole milk were given a diet of yellow corn 20 parts, corn starch 33, chopped timothy hay 30, cane molasses 10, bone meal 2, salt 1 and urea 4 parts, as soon as they would consume dry feed. The calves continued to grow at a fairly normal rate after the milk was removed from the diet at 7 weeks of age. Calves fed the basal diet without urea failed to increase in body weight after milk feeding was discontinued. Digestion trials and nitrogen balance studies were made when the calves were 3 to 4 months of age. Calves fed the low-protein basal diet were in negative nitrogen balance, while calves fed the basal diet plus urea were able to store nitrogen.

One-half of the calves were fed a daily vitamin supplement consisting of thiamin, riboflavin, calcium pantothenate, crystalline vitamin K, pyridoxin, p-amino benzoic acid, nicotinic acid, choline, and crude mixed tocopherols. Supplements of these B-vitamins had no apparent influence upon the growth rate of the calves, the efficiency of nitrogen storage, nor the riboflavin content of the organs and edible meat of the calves.

P7. The Feeding of Korean Lespedeza Seed as a Protein Supplement for Milk Production.*

H. A. HERMAN AND A. C. RAGSDALE, Dept. of Dairy Husbandry, Univ. Mo., Columbia, Mo.

Chemical analyses of Korean lespedeza seed show a striking similarity in protein content to that of the high protein feeds such as cottonseed meal, soybean oil meal, and linseed oil meal, commonly used in balancing dairy rations throughout the middlewest. It is estimated that over 7,000,000 acres of Korean lespedeza is annually grown in Missouri. This legume annually seeds itself and is grown on soils of wide variety over the entire state. Only about 200,000 acres are annually harvested for seed as yet. The annual seed production is approximately 400 lbs. per acre on the average, but may be as much as 800 lbs. per acre. With this vast supply of a relatively cheap source of protein available, and in view of preliminary experiments at the Missouri Station indicating that the ground seed might serve in the rations for lambs and poultry, an experiment of the alternate feeding trial plan was conducted, using two groups of 10 dairy cows each, to determine the value of the ground seed as a substitute for cottonseed and soybean oil meal in the ration of milking cows. The cows in each group were comparable as to body weight, stage of lactation, and daily milk yield.

Each feeding trial consisted of a 40-day period with a 10-day preliminary period. The cows were fed alfalfa hay and sorgo silage as a source of roughage and grain in accord with daily milk production. The average daily milk yield was 35 lbs. per cow on two-times-a-day milking.

The lespedeza seed used was finely ground and mixed into a ration so as to give the same protein content as a ration containing 7½ per cent choice cottonseed meal and 7½ per cent soybean oil meal as the main source of protein.

The seed used gave the following analyses: total dry matter 92.48%, crude protein 34.5%, crude fiber 9.5%, ether extract 7.93%, nitrogen-free-extract 34.13%, ash 6.42%. The grain mixture fed to each group averaged 13.8% crude protein.

In the first 40-day feeding trial following a 15-day preliminary trial, the ten cows fed ground Korean lespedeza seed as a source of high protein averaged 32.68 lbs. of milk daily, whereas the group of cows receiving a ration with the protein supplement made up of equal parts of soybean oil meal and cottonseed oil meal averaged 32.67 pounds daily. At the end of the first 40-day trial, the groups were reversed as to grain ration fed and the group formerly fed Korean lespedeza seed were fed the soybean oil and cottonseed oil supplements. The second trial, following the usual 10-day preliminary period between trials, resulted in the 10 cows receiving the lespedeza seed supplement averaging 27.74 pounds of milk daily. The control group averaged 27.34 lbs. daily. The average per cent of fat in the milk produced by the two groups was the same, or from 3.7 to 3.8 per cent fat.

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* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station Journal Series No. 844.

The amount of grain feed, silage, and hay fed the two groups was approximately equal and was carefully controlled. The cows maintained their body weight and were in good physical condition, on both rations. The ground Korean lespedeza seed mixture was eaten readily and seemed to in no way affect the palatability of the grain ration.

These trials indicate that the proteins of ground Korean lespedeza seed are equal, pound for pound, to the proteins of a mixture of equal parts of cottonseed oil meal and soybean oil meal in the ration of lactating cows.

The use of Korean lespedeza seed as a protein supplement in the usual dairy rations, fed over a long time period, is now under investigation at the Missouri Station.

P8. The Biological Values of Lespedeza, Alfalfa, Corn and Milk Proteins for Growing Dairy Heifers.*

ERIC W. SWANSON, H. A. HERMAN, AND A. C. RAGSDALE, Dept. of Dairy Husbandry, Univ. Mo., Columbia, Mo.

Korean lespedeza has become the major legume forage crop grown in Missouri and several surrounding states. Under favorable conditions a profitable yield of lespedeza seed may also be harvested. The nutritive value of the proteins of lespedeza hay was compared with that of alfalfa hay proteins and an attempt was made to determine if a supplementary effect existed between lespedeza hay proteins and milk and corn proteins respectively. Since lespedeza seed may be used as a protein supplement feed, the biological value of its protein was also investigated.

The biological values were determined with dairy heifers by application of Mitchell's method. Eight fifteen- to eighteen-months-old purebred Holstein heifers were used in the nitrogen metabolism trials. Endogenous urinary nitrogen and fecal metabolic nitrogen were determined by feeding a low protein ration of oat straw, corn starch, sugar, and minerals. Feces and urine were collected separately by a mechanical continuous collecting apparatus, sampled aliquotly each day, and the preserved composite sample was analyzed. All preliminary and collection periods were ten days. The protein content of all rations (except one lot of lespedeza hay) was adjusted to 10% total crude protein by adding sugar and starch.

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* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station Journal Series No. 843.

A preliminary trial early in 1941 with four heifers gave average values of 77% for the biological value of alfalfa protein, 87% for lespedeza hay protein, 76% for corn protein, and 85% for a mixture of corn and lespedeza. Fourteen per cent of the protein of the latter ration was from corn, 85% from lespedeza. No supplementing effect of the corn and lespedeza proteins was apparent.

The trials were repeated, 1941–42, with four different heifers and different lots of hays. Average biological values (percentage of absorbed nitrogen retained by the body) obtained in the second trial were 88.6% for one lot of lespedeza hay, 84.5% for a second lot of lespedeza hay, 73.4% for alfalfa hay, 70.3% for milk protein added to oat straw, 74.0% for lespedeza seed added to oat straw, and 84.1% for a mixture of the first lot of lespedeza hay and dried skim milk such that 33% of the protein was furnished by milk and 66% by the hay. This figure indicates a very slight supplementing effect of the proteins but not a significant one.

The digestibility of the protein of the lespedeza hay rations was much lower than that of any of the other rations. There was an inverse relation between the biological values and the digestibility of the nitrogen of the rations which resulted in practically identical net utilization (biological value times digestion coefficient) of the protein of all the rations. The average percentage of nitrogen intake stored was 22.6% on the first lespedeza hay ration, 24.6% on the second, 20.8% on the alfalfa hay, 27.6% on the lespedeza and milk ration, 22.2% on the milk ration and 21.9% on the lespedeza seed ration. There was no statistically significant difference between any two rations in percentage of ingested nitrogen stored.

The results indicate that although the lespedeza hay protein was not digested as well as alfalfa, corn, milk protein, or lespedeza seed protein, the digested protein was utilized to a greater extent by growing dairy heifers with the result that the net utilization was the same for all the rations as they were fed in this investigation.

P9. A Comparison of Acetic Acid, Fed as Triacetin, with Glucose as a Nutrient in Feeds. T. B. McManus and C. B. Bender, N. J. Agr. Expt. Sta., New Brunswick, N. J.

Two experimental rations, the first containing 15% glucose and the second an equicaloric amount of acetic acid in the form of triacetin, were fed to albino rats using the paired feeding method of controlling food intake. The efficiency of the two rations was compared through several measurements.

A six-week study of the growth rates of the rats was made. This showed that the rations were equally efficient in maintaining the animals and supporting growth. A study of the heat production of the animals receiving the two rations showed no difference in their effect on the rate of metabolism. The metabolizable energy of the two rations was determined. The gross energy of the glucose-containing ration was 94.5% metabolizable and that of the triacetin containing ration was 94.3% metabolizable. A study of the loss of volatile acids in the urine of the animals showed that the acetic acid of the triacetin-containing diet was retained.

The results show that the two rations were equivalent. Acetic acid, fed as triacetin, was absorbed from a balanced ration and retained by the animal. It was as effective in supporting growth as an equicaloric amount of glucose and it had no greater effect on the specific dynamic action of the ration than did glucose.

It is concluded that acetic acid, in the form of triacetin, was utilized with a degree of efficiency equal to that of glucose.

P10. Ruminal Gases in Normal and Bloated Animals.

T. M. OLSON, S. Dak. Agr. Expt. Sta., Brookings, S. Dak.

Bloat in cattle has been known among livestock men for a long time. It has also been recognized that some plants, particularly legumes, cause bloat more readily than non-legumes.

The immediate cause of bloat is assumed to be a rapid formation of gases in the rumen. The assumption is that the gases accumulate in the rumen to the point where the extension of the rumen exerts sufficient pressure on the diaphragm to interfere with the normal functioning of the heart and lungs. The gases concerned were thought to be carbon dioxide and methane, although no published data were available until very recent years to indicate that these gases had been determined in a laboratory.

Inasmuch as the cause of death from bloat is attributed to excessive pressure on the vital organs it is important to know what this pressure is, and whether it is sufficient to produce the results claimed. Pressures have been determined at the South Dakota Station on a number of bloated animals and animals which have died from bloat, and found to range between 60–70 mm. of mercury above atmospheric pressure. Other animals have been insufflated with air from a compressor up to 90–100 mm. of mercury with no ill effects except discomfiture of the animal. As soon as the pressure was released no after effects were observable.

In more recent years analyses have been made of ruminal gases, which indicate that in addition to carbon dioxide and methane two additional gases, viz., carbon monoxide and hydrogen sulphide, are also present in the ruminal gases. These gases are present in varying amounts in the rumen of bovine on normal dry feeds. It has also been determined that the quantity of these gases will vary with individuals on the same feeds, and with the kind of feeds eaten by the animal.

Recent experimental work indicates that when animals bloat, there is considerable increase in hydrogen sulphide gas, as compared to animals on dry feed. The amount of hydrogen sulphide gas may be ten to twenty times that of animals on dry feed.

Toxicologists have known for some time that hydrogen sulphide gas is highly toxic. Small amounts of this gas when absorbed into the blood stream is fatal. Recent laboratory determinations have demonstrated that alfalfa under certain stages of growth is very high in hydrogen sulphide gas. This seems to indicate that this gas plays a significant role in the cause and death of bovines from bloat.

P12. Further Nutritional Studies on Calf Scours.

NORMAN S. LUNDQUIST AND PAUL H. PHILLIPS, Univ. Wis., Madison, Wis.

Previous work at this Station has shown that calf "scours" in many cases is of nutritional origin. Recent studies have yielded data which further clarify the early dietary requirements of the calf. New-born Holstein calves can be satisfactorily raised on a properly fortified skim milk diet without access to colostrum. This has not been accomplished with Guernsey calves. Colostrum feeding for three days followed thereafter by a properly fortified skim milk diet was found to be a satisfactory diet for the calves of all breeds used. It has been found that ascorbic acid is necessary in adequate amounts in the early diet of the calf to prevent and control navel infection. Ascorbic acid can be administered per os for the first 10–12 days of life, thereafter, it must be injected to be effectively recovered in the blood stream. Blood plasma ascorbic acid in the normal calf diminishes from birth to the third week of life, then gradually returns to a normal level of approximately .3 mg. %. These observations seem to be closely related to the initial functional activity of the paunch. These studies further emphasize the point that both vitamin A and certain members of the vitamin B complex are required by the calf for the control and prevention of scours. In nutritional scours, certain members of the vitamin B group are indicated when the blood plasma vitamin A concentration is above .10γ/cc. and vitamin A administration is required either alone or in combination with these B vitamins when the blood plasma vitamin A is below this level. It has been shown that pantothenic acid alone was ineffective. The addition of nicotinic acid (alone) to the milk diet was distinctly beneficial for the control of scours when adequate vitamin A was present.

P13. Factors Affecting the Vitamin A and D Potency of Alfalfa Hay.*

G. C. WALLIS, Dairy Dept., S. Dak. Agr. Expt. Sta., Brookings, S. Dak.

The importance of good quality roughages in maintaining the health and productiveness of livestock is becoming increasingly evident. In this study the factors and methods concerned with (1) the rapidity of moisture loss, (2) the conservation of vitamin A values, and (3) the development of vitamin D activity in the curing and storing of alfalfa hay are under investigation. When these fundamental principles are understood, effective methods of making and keeping alfalfa hay and other roughages of high nutritive quality can be devised and evaluated. Thus far studies have been made on a third cutting of alfalfa cured in the swath and windrow, and on two second cuttings each cured simultaneously in swath, windrows, and cocks. Some studies have been made on losses during winter storage under hay mow conditions.

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* South Dakota Agricultural Experiment Station, Journal Series 164.

According to the present information when hay is cured in the swath and in small windrows from immediately after cutting the moisture loss is most rapid from the swath for only about the first half day. By the end of the day there is less moisture in the small windrow and this relationship continues from then on. Most rapid drying can be secured by leaving hay in the swath for a half day, then raking it into small windrows. Turning the windrow after the hay is $\frac{1}{2}$ to $\frac{2}{3}$ dry may still further hasten the drying process.

For the first day there is not so much difference in the rate of destruction of carotene in the swath and small windrow, but after that the destruction in the swath is more rapid so that by the time the hay is ready to haul the small windrow may have up to twice as much carotene as that cured in the swath. Carotene analyses of hay which was cured out completely in the cock without spoiling are not yet available for comparison.

Vitamin D analyses are available for the third cutting samples and for one of the second cutting series of samples. This evidence indicates that it takes from 2 to 4 days of good sunshine exposure to develop the maximum amount of vitamin D in the hay. The vitamin D development was very similar in the swath and small windrow but there was little or no additional development in the cocks beyond the .23 I.U. of vitamin D per gram of air dry material at the time of cutting. There is some evidence that light showers tended to destroy some of the vitamin D which had been previously developed in the hay. The third cutting hay cured under September sunshine developed up to 5 International Units of vitamin D per gram whereas the second cutting cured under similar conditions of July sunshine failed to develop more than 1 International Unit per gram. Whether this was due to the presence of more activatable material in the third cutting hay or to some other cause and whether or not it will prove to be the general experience is not known at the present time.

In 5 months of storage in the mow swath cured hay with a carotene content of 88 micrograms per gram of dry matter dropped to 16 micrograms and hay cured in a small windrow dropped from 158 to 28 micrograms per gram. There was a further slight loss in both hays during the next three months of storage. There was some evidence of a slight loss in vitamin D during the first 5 months but none in the succeeding 3-month period.

P14. The Vitamin A and Carotene Content of the Blood Plasma of Calves from Birth to Four Months of Age. L. A. Moore,* Maryland Agr. Expt. Sta.

The question of the vitamin A requirement of calves from birth to four months of age has not been widely investigated although this is the most difficult period of the calf's life. It was thought that the determination of the vitamin A and carotene content of the blood plasma of calves of this age group under the usual conditions of herd management might throw some light on the subject from the practical point of view.

Samples of blood were collected from 12 calves before they had colostrum and at 24-hour intervals up to one week of age. Samples were also taken every two weeks on about 50 dairy calves and 20 beef calves up to four months of age.

The vitamin A and carotene content of the blood plasma of a new born calf is very low. These data show that after taking colostrum the plasma vitamin A of dairy calves increased markedly and the carotene increased slightly up to the third day when the calves were removed from the cow.

The blood plasma of Jersey and Guernsey calves from birth to 4 months of age contained as much vitamin A as that of Holstein and Ayrshire calves.

Under the usual conditions of herd management it was found that the blood plasma from beef calves contained twice as much vitamin A as the blood plasma from dairy calves. This raises the question as to whether dairy calves raised under a system of reduced milk intake as is usually practiced on most dairy farms receive sufficient vitamin A and carotene from birth to four months of age.

It was found that the feeding of a good quality of lespedeza hay very markedly increased the vitamin A and carotene content of the blood plasma of dairy calves after about one month of age above that of calves receiving a good grade of alfalfa or clover and timothy.

P15. Vitamin C in Dairy Cattle Nutrition.† G. C. Wallis, Dairy Dept., S. Dak. Agr. Expt. Sta., Brookings, S. Dak.

In the course of our studies on the vitamin D deficiency of dairy cows some symptoms were noted which indicated the possibility of a complicating vitamin C deficiency. As these animals had been on a ration which was also low in vitamin C for three years or so it was thought advisable to investigate this possibility.

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* The data to be presented were collected from the college dairy and beef herds of the Michigan State College and the dairy herd of the University of Maryland.

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† South Dakota Agricultural Experiment Station, Journal Series No. 163.

The vitamin C in the blood plasma and the milk was determined twice monthly for a period of a year to see if the vitamin was available for physiological purposes and for secretion into the milk. For comparative purposes, similar determinations were made on the three other groups of cows receiving rations which provided increasing amounts of vitamin C. One of these groups consisted of experimental cows receiving alfalfa hay and a grain mixture and the other two groups were taken from the college herd. Of the latter, one group received alfalfa hay, corn silage, and a grain mixture while the other received pasture supplemented with grain which would supply a rich source of vitamin C. The indophenol titration method was used for these vitamin C determinations.

The vitamin C content of the blood plasma varied on the average between 0.3 and 0.4 milligrams per 100 ml. with no appreciable differences between the groups. The experimental cows on the vitamin C low ration were even a little higher in this respect than the cows receiving a large intake on pasture. The vitamin C content of the milk was also quite constant at about 18 to 20 milligrams per liter and showed no significant differences between groups of cows or between morning and evening samples.

Six vitamin C balance trials have also been carried out on the experimental cows receiving the vitamin C low ration, and varying in milk flow from dry cows to those in heavy production. In this case the vitamin C in the feed, feces, and urine was determined by the use of a photoelectric colorimeter and the indophenol dye. In all cases the outgo was decidedly larger than the intake. The outgo in the urine was very small in comparison to that of the milk and feces. For instance, in one case the daily intake was 73 milligrams and the outgo in 49.5 pounds of milk was 526 milligrams, in the feces 703 milligrams, and in the urine only 19 milligrams. The total outgo was 1248 milligrams. Such decidedly large outgoes as compared with the intake over long periods of time gives strong evidence of an active vitamin C synthesis by dairy cows. The large outgo in the feces compared with the urine suggests a synthesis along the gastro-intestinal tract although the appearance of appreciable amounts of vitamin C in the milk indicates a rather free absorption into the blood stream with possibly a re-excretion into the intestinal tract. Of course it could be synthesized elsewhere in the body and be excreted via the intestinal tract rather than the kidneys.

P16. Carotene (Provitamin A) Requirements of Dairy Cattle for Conception.

A. H. Kuhlman and W. D. Gallup, Oklahoma A. and M. College, Stillwater, Okla.

The minimum daily carotene requirement of dairy cows for normal reproduction appears to be between 40 and 45 micrograms per pound body weight. The carotene requirements of first-calf heifers may be somewhat higher than those of older cows since the former animals need carotene for growth in addition to that needed for maintenance and reproduction. Apparently the amount of carotene needed for lactation is not in excess of that required for successful reproduction. The level of carotene intake required to maintain fertility of cows is not known.

Records of the intake of carotene previous to breeding are available for 27 grade Jersey females for a total of 80 services and 58 conceptions. With the exception of several cases in which Puratene (a commercial carotene supplement) was also used, prairie hay was the sole source of carotene. The average daily intake of carotene per pound body weight for the 90-day period preceding service varied from 20 to 353 micrograms.

Forty-three of the 58 conceptions resulted from the first service, 12 from the second, and one each from the third, fourth, and sixth services. For the entire group 1.38 services were required per conception. In 21 cases in which cows had an average daily intake of 20–39 micrograms carotene per pound body weight for the 90-day period preceding service, 1.99 services were required per conception. When the daily carotene intake was 40–59 micrograms, 60–99 micrograms, and 100–353 micrograms, 1.35, 1.15, and 1.23 services per conception were required respectively in 23, 15, and 21 cases.

While the results obtained do not establish the minimum carotene requirements for conception, they do indicate that a very satisfactory conception rate was obtained when the carotene intake was at the same level as that required for normal calving.

P17. The Relation of Nutrition to Breeding Performance in Dairy Bulls.

I. R. JONES, J. R. HAAG, AND R. W. DOUGHERTY, Oregon Agr. Expt. Sta., Corvallis, Oregon.

In a study to determine the effect of the ration on fertility, 18 bulls were fed experimentally from birth to 31 to 40 months of age as follows:

Group A. Four bulls. Good quality alfalfa hay after seven months of age and free access to disodium phosphate.

Group B. Two bulls. Same ration as Group A supplemented daily with 1 pound each of skimmilk powder and oat groats.

Group C. Four bulls. After 3 months of age weathered hay bleached until practically devoid of green color and about 3 pounds daily of a mixture of oats, barley, skimmilk powder, linseed meal, wheat bran, bone flour and salt.

Group D. Two bulls. Same ration as Group C supplemented daily with approximately 2 ounces of salmon oil containing about 5000 U. S. P. units of Vitamin A per gram.

Group E. Four bulls. Plain dried beet pulp as the only roughage. After 90 days about 3 pounds daily of a mixture made up of 66 per cent corn gluten meal, 30 per cent skimmilk powder, 2 per cent each of bone flour and salt. Approximately 10 cc. of salmon or cod liver oil was fed daily.

Group F. Two bulls. Same ration as Group E supplemented with 10 cc. of wheat germ oil fed on the grain once weekly.

Salt and bone flour was available to the bulls at all times and iodine was supplied to all animals once weekly in the form of potassium iodide solution.

Group B animals made normal growth followed by Groups E and F whose gains were almost identical and only slightly below normal, Group A from 10 per cent to 15 per cent below normal until 2 years of age, Group D averaging about 20 per cent below normal from 6 to 18-month period and Group C averaging 25 per cent below normal during the 6 to 18-month period. After about 18 months of age the animals below normal grew at an accelerated rate so that at the time of disposal growth differences between the groups were largely eliminated.

The results of 299 semen examinations showed considerable variations among the individual animals and with the same animal at different times. Some of the bulls showed remarkably constant sperm counts, motility ratings and kind and percentage of abnormal forms. Several animals would have been rated as questionable breeders on the basis of low sperm counts and volume, poor motility, variable pH readings and 25 to 40 per cent abnormal forms. However, there appeared to be no particular relation between so-called low quality sperm and the rations fed.

The fertility of 17 of the bulls was proven by service to 303 cows. At the present writing pregnancy and calving records are complete on 181 cows which averaged 1.40 services per conception. Of the calves born 168 were normal, 5 were weak or dead at birth and 8 pregnancies resulted in abortions.

A study of the individual bull and group records shows some individual but no large group variations from the average. Only one bull averaged more than 2 services per pregnancy. Apparently a ration adequate for fairly normal growth to 3 years of age in a dairy bull is satisfactory for normal reproductive performance.

Other data taken during the course of the investigation included blood carotene values, blood phosphorus determinations and ascorbic acid content of the semen. Following slaughter the testes of the bulls were sectioned. Preliminary histological studies indicate no outstanding differences in spermatogenesis and interstitial tissue development with the bulls fed different rations.

P18. Some Preliminary Results of Feeding Chloretone to Bulls.

E. C. Scheidenhelm, A. L. Bortree, C. F. Huffman, and C. F. Clark, Michigan State College, East Lansing, Mich.

Sterility in dairy bulls has been a vital problem to dairymen for years.

In 1941 Phillips and coworkers at the University of Wisconsin reported that the subcutaneous injection of ascorbic acid into sterile bulls resulted in marked increase in breeding efficiency. Bortree and associates in 1941 found that the feeding of chloretone to cattle increased the blood plasma ascorbic acid level. The feeding of 5 grams of chloretone per day resulted in an increase in plasma ascorbic acid, usually within 5 days.

In the present investigation the effect of feeding chloretone at various levels for different periods of time was studied as well as the effect upon sex interest and breeding efficiency of sterile bulls.

Two 1800-pound bulls were each fed 10 gms. of chloretone per day. One of these bulls lost its sense of balance after 38 days due to the anesthetic effect of chloretone. The other bull was normal. The amount of chloretone fed per day to these two bulls was reduced to 5 gm. at this time and continued for a period of 4 months with no ill effects.

Three slow breeding bulls were used to determine the effect of feeding chloretone upon sex interest. The first bull required 10 to 15 minutes to serve a cow before treatment. There was continuous improvement in sex interest after chloretone feeding, until at the end of the fourth month this bull served a cow immediately. The second bull which failed to serve a cow during a one-hour period before treatment, responded to chloretone feeding as was indicated by a service in 5 minutes following 3 weeks' treatment. A third bull that required 10 to 15 minutes per service prior to chloretone feeding, bred a cow in 3 to 4 minutes after 3 weeks' treatment.

The effect of chloretone administration upon the number of services per conception was investigated using three bulls. The first bull required 5.33 services per conception during 7 months previous to the feeding of chloretone. During the 6 weeks following chloretone feeding the number of services per conception was 1.50. The second bull which showed 8 services per conception during a 2-month period prior to treatment, required 2.33 services per conception for the same period following chloretone treatment. In case of the third bull 8.5 services per conception were required during the 2 months prior to treatment, while only 2.66 services per conception were needed during the three months following the feeding of chloretone.

Additional studies on these problems are in progress and will be reported later.

P19. Effect of Amphyl on Bull Sperm.

H. O. DUNN, C. E. SHUART, AND O. F. GARRETT, N. J. Agr. Expt. Sta., New Brunswick, N. J.

Amphyl is the trade name of a concentrated disinfectant consisting of a mixture of p-chloro-s, m-dimethyl hydroxybenzene, p-tert amyl hydroxybenzene and neutral soap dissolved in alcohol, glycerol and water. It has a phenol coefficient of 10 but is practically non-corrosive to animal tissue.

In a search for a substitute method for heat sterilization of the equipment used in artificial insemination, Amphyl was tried. In 10 trials it was found that possible disinfectant residues left in the glass tubes, used for receiving the ejaculate, after treatment with Amphyl had no effect on the longevity or motility of the sperm. To make the test more severe, Amphyl, in various concentrations, was added directly to semen and the longevity and motility of the sperm were observed. The semen was usually diluted with an egg yolk-phosphate buffer solution.

Addition of Amphyl to undiluted semen destroyed all sperm within 30 seconds. Addition of the disinfectant to semen diluted only with the phosphate buffer rapidly destroyed the sperm. When 0.2% to 0.5% of Amphyl was added to semen diluted with the egg yolk-phosphate buffer solution, however, the sperm lived from 4 to 9 days longer than sperm in the same diluted semen but containing no Amphyl. In one trial, for example, all sperm in the control semen were non-motile by the sixth day, whereas, 30% of the sperm in the Amphyl-treated semen were still motile on the thirteenth day.

The rate of the destruction of bacteria in the control semen was about the same as that in the Amphyl-treated semen.

P20. The Relation of Morphology to Fertility in Bull Semen. G. W. Trimberger and H. P. Davis, Nebr. Agr. Expt. Sta., Lincoln, Nebr.

The morphological characteristics of the spermatozoa in the semen from 24 dairy bulls of the Holstein, Jersey, Guernsey, and Ayrshire breeds used for service in the University of Nebraska dairy herd were observed regularly at monthly intervals over a period of several years for a total of 376 months. The numbers of normal and abnormal sperm cells were determined by counting one thousand cells after they had been stained. When all samples were included, the 24 bulls averaged 790.5 normal cells per thousand with a range from 276 to 968 for individual samples and from 373 to 903.4 from the lowest to the highest average for a bull. The breeding efficiency was 57.76 per cent conception in the 483 cows bred during the experimental period.

No differences were found in the number of normal sperm cells in semen samples obtained by the massage method per rectum compared to those obtained with an artificial vagina from the same bulls nor was there any definite relation between any particular abnormal form and decreased breeding efficiency. Free heads or heads without tails was the most common abnormality found among the cells, with broken tails and looped tails ranking next in order. A monthly tabulation for seasonal variation showed some fluctuation in the number of normal spermatozoa but no significant differences were found.

Bulls with over 900 normal sperm cells per thousand had significantly better breeding records than those below this high level, and 8 of the 24 bulls sampled or 33.33 per cent had a month or more with over 900 normal cells per thousand although only 11.44 per cent of all samples were of this high quality. A total of 71 cows were inseminated with semen taken from bulls during a month in which they had over 900 normal sperm cells per thousand and 53 or 74.65 per cent of these cows conceived. From 76 cows bred with semen from bulls during months in which they had from 851 to 900 normal cells per thousand, the result was 48 or 63.16 per cent conceptions. There was relatively little difference between the breeding records of bulls with 500 to 850 normal sperm cells per thousand divided by class intervals of 50 and all but one class averaged 50 per cent or more conceptions, but the average for 325 cows bred was 51.08 per cent conceptions. Bulls with less than 500 normal cells per thousand had very poor breeding records.

Comparison of the first and second ejaculate showed a slight advantage in favor of the second but no significant differences when the previous service had been within 20 days. A marked and significant difference was found if the previous service was not within the 20 day limit. This difference was more pronounced when the previous sample had been taken between 20 and 30 days than when the time interval between samples was over 30 days because after a long rest period more than two ejaculates are necessary to return to normal. This suggests the advisability of taking two or more services from a bull if he has not been used for several weeks.

P21. Studies of Respiration Rate of Dairy Bull Spermatozoa.* RAY E. ELY, Dept. of Dairy Husbandry, Univ. Mo., Columbia, Mo.

The relationship between the average number of services per conception and respiration rate of dairy bull spermatozoa has been reported. Other characteristics of an ejaculate, such as initial motility, percentage of abnormal spermatozoa, and survival on storage have been correlated with the bull's fertility or breeding record.

In this investigation a study was made of the oxygen consumption of dairy bull spermatozoa and the relation to other characteristics of the ejaculate. The bulls used represent wide variation in their breeding records and physical and chemical characteristics of the ejaculates.

Samples were collected with an artificial vagina with precautions to avoid temperature shock and were held at a standard temperature until the respiration determinations were begun. In all cases the first readings were taken between 90 and 120 minutes after the ejaculate. A modified Barcroft-Warburg respirometer at 37° C. was used for measurement of oxygen consumption. Measurements were made in a buffered seminal fluid medium either by dilution with phosphate buffer or removal of one-half the seminal fluid after centrifugation and making up to volume with phosphate buffer. A staining technique for differentiation of living and dead spermatozoa was used for calculating the results on the basis of number of living spermatozoa as well as total number of spermatozoa.

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* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station Journal Series No. 842.

Samples maintaining a 2 motility rating an average of 156 hours on storage at 40° F. had over twice the oxygen consumption of samples surviving less than 24 hours on storage. Intermediate samples in length of survival on storage were intermediate in volume of oxygen consumption. There was a closer relation between the survival time of individual samples and respiration rate than of the average survival time of a given sire's ejaculates and his average respiration rate.

Three groups of samples according to their per cent of abnormal spermatozoa had a decreasing oxygen consumption with increasing numbers of abnormal spermatozoa.

Centrifuging lowered the oxygen consumption of the sperm suspension as compared to control samples; however, the number of living spermatozoa was also decreased, while oxygen consumption per billion living spermatozoa remained essentially the same.

Addition of seminal fluid caused a stimulation of oxygen uptake above the rate of washed spermatozoa in phosphate buffer. The increase was much greater than accounted for by the oxygen uptake of the seminal fluid alone.

The oxygen uptake of the seminal fluid represented quite wide ranges in the percentage of total oxygen consumption of sperm suspension and could not be accurately estimated from measurements of the buffered semen samples.

P22. The Breeding Efficiency of Dairy Bulls Used Both Artifically and Naturally.*

E. R. Berousek, Dept. of Dairy Husbandry, Univ. Mo., Columbia, Mo.

A survey of the literature concerning the artificial breeding of over 10,000 dairy cows indicates that approximately 1.7 services per conception were required as compared to approximately 2.2 services per conception for approximately the same number of cows in various herds where natural breeding was practiced. The records concerning artificial breeding cover only the past four years, whereas those for natural breeding are taken from reports dating back as early as 1900. In order to compare natural vs. artificial breeding efficiency, half the Missouri Station dairy herd was bred naturally and the other half artificially for a 2-year period (1937–1939). During this period artificial breeding required 1.59 services and natural breeding 1.66 services per conception.

The study reported herein includes 14 bulls used both artificially and naturally—8 Holsteins, 5 Jerseys, and 1 Guernsey. Only records of cows or heifers which have proved to be fertile are included in this summary. A few animals suffering genital abnormalities, and failing to conceive irrespective of the sire or breeding method used, have been eliminated from the averages.

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* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station Journal Series No. 841.

The eight Holstein bulls settled 209 cows with 347 natural services with an average of 1.66 services per conception. Semen from the same bulls used to inseminate 127 cows resulted in 67 pregnancies or 1.89 services per conception. The average service rate per bull ranged from 1.33 to 2.02 for natural breeding and from 1.00 to 5.00 artificially. One bull was used 146 times naturally and settled 90 cows with an average service rate of 1.62. The same used 67 times artificially settled 34 cows with an average of 1.97 services per conception. Another bull used only four times naturally settled three cows. Semen from the same bull was used to inseminate five cows and all five of them conceived. One young bull was used 9 times artificially and all 9 cows were settled. He was never used naturally and is not included in this report.

One hundred and fifty-one cows were bred naturally by the five Jersey bulls and 95 of them conceived for an average of 1.59 services per conception. One hundred and six cows were artificially inseminated with semen from these bulls and 49 settled, requiring 2.16 services per conception. The service rate per conception ranged from 1.38 to 4.66 for naturally bred and 1.32 to 4.25 artificially.

The Guernsey bull served 49 cows with 31 pregnancies resulting, or 1.58 services per conception. Twenty cows bred artificially, of which 12 conceived, required 1.67 services per conception.

The 14 bulls had a total of 547 services with 335 conceptions when used naturally and 253 inseminations with 128 conceptions when used artificially. The average number of services per conception was 1.63 and 1.98, respectively. There were many variations per individual service rate. Four bulls had a lower service rate per conception when used artificially than when used naturally as compared to nine bulls whose lowest service rates were obtained when used naturally. One bull had the same service rate per conception for each method.

The lower efficiency of artificial breeding is not considered significant because in many cases semen stored for 24 to 72 hours was used. A few of the bulls used in the herd in recent years were old proved sires low in fertility and during this time most of the breeding has been done by artificial means. All factors considered, it is concluded that there is little difference in the service rate of natural and artificial breeding.

P23. A Comparison of Artificial vs. Natural Service in Heifers When Bred to the Same Sire.

C. E. Shuart, O. L. Lepard, and J. W. Bartlett, N. J. Agr. Expt. Sta., New Brunswick, N. J.

Forty-four virgin Holstein and Guernsey heifers were alternately selected within each breed for artificial or natural service as they reached 18 months of age. Only heifers which had previously shown normal oestrus cycles were selected.

The sires were virgin Holstein and Guernsey bulls. All heifers were served naturally or artificially to these bulls according to their grouping and breed. The breeding was limited to one service or one ejaculate unless four or more days had elapsed since a sire had been used. In this case two services were allowed or two ejaculates collected. If more than one animal was bred naturally to the same sire on the same day, six hours elapsed between services. Likewise if one animal was bred naturally and another artificially inseminated, six hours elapsed between the service and collection. All inseminations were made immediately after collection and examination with undiluted semen. Examinations for pregnancies were made at approximately sixty days after the last service.

The study shows a significant difference in conception rate between the two groups. The 22 animals artificially inseminated required 24 services, giving a conception rate of 1.06 (services per conception) with 90.6 per cent conceiving on first service. The 22 heifers bred naturally required 37 services, giving a conception rate of 1.67 (services per conception) with 63.6 per cent conceiving on first service.

P24. The Availability of Carotene in Alfalfa Hay as Compared with Carotene in Oil. J. H. HILTON, J. W. WILBUR, R. J. WESTFALL, AND S. M. HAUGE, Purdue Univ., Lafayette, Ind.

Two groups of two Guernsey cows each were used in feeding trials to determine the relative availability of carotene in dehydrated alfalfa hay as compared with carotene dissolved in oil. The body stores of vitamin A in the cows were depleted in a preliminary feeding period by feeding a vitamin A deficient ration. In successive feeding periods, the carotene of the hay and the carotene in the oil' were equilibrated at levels of 130, 200, 300 and 200 milligrams daily during the respective feeding periods.

Biological assays of the milk fat secreted by the two groups of cows on the respective rations indicate that dairy cows can utilize carotene in plant tissues as readily as isolated carotene.

P25. The Cause of the Initiation of Lactation at Parturition.* JOSEPH MEITES AND C. W. TURNER, Dept. Dairy Husbandry, Univ. Mo., Columbia, Mo.

In most animals the mammary glands reach a full state of development during pregnancy, but usually there is little or no milk secretion until after parturition. The study of the lactogenic hormone in the pituitary of numerous animals during pregnancy and lactation has consistently revealed that the lactogen content of the pituitary remains relatively low during pregnancy, but increases sharply following parturition. The conclusions were drawn, therefore, that (1) the lactogen content of the pituitary was intimately associated with mammary secretion; (2) the low lactogen content of the pituitary during pregnancy probably accounted for the relative absence of milk secretion during this period; and (3) at parturition, some activating factor was responsible for the increase in the lactogen content of the pituitary and the consequent onset of lactation.

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* Contribution from the Department of Dairy Husbandry, Missouri State Agricultural Experiment Station Journal Series No. 837.

Experimentally, the administration of estrogen has been found to greatly increase the lactogen content of and secretion by the pituitary, and also to initiate milk secretion in suitably prepared mammary glands. Small or moderate amounts of estrogen were shown to be more effective in these respects than large dosages of hormone. By administering estrogen for only 5 days into mature female rats or into immature guinea pigs of either sex, it was found possible to increase the lactogen content of the pituitary by more than 200 and 400 per cent, respectively, or to levels higher than or equal to the amount of lactogen present in the pituitaries of these animals after parturition.

During the course of pregnancy, despite the greatly increased amounts of estrogen which are known to be present, there is little or no increase in the lactogen content of the pituitary. Apparently, some factor overrides the stimulating effect of estrogen on the pituitary during this period. This is not entirely unexpected, however, since other physiologic effects of estrogen are also overriden during pregnancy. Thus, (1) estrus is suppressed, (2) ovulation is inhibited, and (3) the uterine musculature remains relatively quiescent. There is good reason to believe that it is progesterone which overrides all these normal physiologic effects of estrogen during pregnancy. Thus, if the corpora lutea are removed during advanced pregnancy in some animals, the fetus or fetuses are expelled, ovulation and estrus set in, and milk secretion is initiated. On the other hand, if the corpora lutea are maintained, or if progesterone is administered, pregnancy can be prolonged beyond the normal span of time.

It is the belief of the writers, therefore, that during pregnancy, progesterone overrides the stimulating effect of estrogen on the lactogenic hormone of the pituitary, but at parturition with the removal of progesterone from the organism, estrogen is able to instigate a rapid increase in the lactogen content of the pituitary and thus initiate lactation. The experimental basis for this theory will be presented.

On the basis of this theory, it appears probable that moderate doses of estrogen administered after parturition may prove beneficial in cases of hypogalactia in animals, provided that such abnormalities are caused by a deficiency of lactogenic hormone.

P26. Prehypophyseal Hormone (Mammogen) Control of Mammary Development.* E. T. GOMEZ, Division of Nutrition and Physiology, Bureau of Dairy Industry, U.S.D.A., Washington, D. C.

Stimulation of growth of the duct system of the mammary glands of hypophysectomized-castrated guinea pigs has been induced either by implanting (2 cases) or injecting (8 cases) 20 to 50 mg. of fresh prehypophyseal gland tissue from male or female guinea pigs. The material was injected in saline suspension, the dose being apportioned over a period of 10 days. The total material obtained by extracting the same amount of fresh prehypophyseal gland tissue with a warm mixture of absolute ethyl alcohol (8 parts) and ether (U.S.P., 2 parts) was injected, similarly apportioned, in 10 daily administrations. This resulted in growth of the mammary glands, equaling that of early pregnancy, in hypophysectomized-castrated guinea pigs of either sex (10 cases). The extract showed no histological evidence of reparative effect upon the atrophied thyroid and adrenals of the test animals; there was no body growth-promoting effect; and, in the females, cornification of the vaginal epithelium was not manifest.

Administered in dosages equivalent to 5 times (100 mg.) the above minimum effective dose level of fresh prehypophyseal glands (20 mg.), the alcohol-ether extracted glands showed no mammary gland growth-promoting activity (mammogenic) in hypophysectomized-castrated guinea pigs (4 cases).

Ten mg. of an impure preparation which contained the lactogenic hormone, when administered daily for 10 to 15 days, either alone (2 cases) or in combination with 75 I.U. of estrone (2 cases) or with 5 mg. of progesterone (2 cases) or 5 mg. of desoxycorticosterone (2 cases), did not cause growth of the mammary glands of hypophysectomized-castrated male or female guinea pigs.

More recently, it has been found that the daily administration of 25 mg. of the lactogenic hormone alone for 10 days causes a slight growth stimulation of the duct system of the mammary glands of hypophysectomized-castrated guinea pigs. The duct growth response was improved when 75 I.U. of estrone was administered daily in addition to the lactogenic hormone treatments (2 cases).

The response noted above with 25 mg. was not obtained, either with or without estrogen, when the lactogenic hormone was pre-treated with warm alcohol-ether mixture (2 cases) although the preparation still possessed lactogenic activity. The original as well as the alcohol-ether treated lactogenic hormone preparation was found to have, in addition to its lactogenic property, appreciable thyrotrophic, gonadotrophic, and adrenotrophic activities.

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* This research was supported by an appropriation from Bankhead-Jones funds (Bankhead-Jones Act of June 29, 1935).

The animals used in the above experiments were hypophysectomized and castrated at 6 weeks of age, when they weighed from 150 to 200 g. The treatments with all these operated animals were started 60 to 100 days after hypophysectomy. The mammary gland growth was determined by study of the whole mounts of stained and dissected glands.

P27. The Effect of Adrenalectomy on the Lactogenic Hormone and Initiation on Lactation.*

J. L. TRENTIN, J. MEITES, AND C. W. TURNER, Dept. of Dairy Husbandry, Univ. Mo., Columbia, Mo.

It has become well established that the adrenals are essential for normal lactation. It has not yet been adequately determined, however, whether the injurious effects of adrenalectomy upon lactation are due entirely to the abnormalities which are known to arise in salt, water, and carbohydrate metabolism, or also because of detrimental effects on the lactogenic function of the pituitary. It was to shed some light on this second possibility that the present investigation was undertaken.

Evidence has been presented elsewhere showing that (a) experimentally, estrogen can increase the lactogenic potency of the pituitary and initiate lactation, and (b) that estrogen is probably the active agent responsible for the natural increase in pituitary lactogen and initiation of lactation at parturition. Answers were sought, therefore, to the following questions: (1) What is the effect of adrenalectomy on the normal lactogen content of the pituitary? (2) Does adrenalectomy affect the ability of estrogen to augment the lactogen content of the pituitary? (3) Can adrenalectomy during pregnancy prevent the normal rise in pituitary lactogen and initiation of lactation which occur at parturition?

The following results were obtained. Fifteen normal female albino rats contained an average of 5.05 Reece-Turner Units of lactogenic hormone per pituitary, while two groups of adrenalectomized rats (total of 33) contained an average of 3.91 and 3.75 R-T.U. of lactogen, respectively. This significant reduction in pituitary lactogen following adrenalectomy may be due to (a) a direct effect on the pituitary, (b) suspension of the estrus cycle, or (c) reduction in food intake.

The administration of 1000 I.U. of estrone into 10 intact female rats increased the average lactogen content of the pituitary to 15.75 R-T.U., or about 212 per cent, while the same amount of estrone injected into 14 adrenalectomized rats increased the average lactogen content of the pituitary to only 10.87 R-T.U., or about 115 per cent. Although the results obtained on adrenalectomized guinea pigs were less conclusive, the available data indicate that estrogen injected into such animals elicits the same increase in pituitary lactogen as in intact guinea pigs.

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* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station Journal Series No. 836.

Albino and white hooded rats which were adrenalectomized during the last week of pregnancy showed the same increase in pituitary lactogen 48 hours after parturition as did intact parturient rats. Milk was present in the mammary glands of the adrenalectomized mother rats and also in the stomachs of their living young, but not in amounts equaling that found in intact rats. It is concluded, therefore, that the failure of rats adrenalectomized during pregnancy to lactate sufficiently following parturition is not due to a deficiency of lactogenic hormone in the pituitary. This conclusion is supported by the fact that lactation cannot be initiated or maintained in adrenalectomized animals by administering lactogenic hormone.

P28. The Influence of Thyroxine upon the Stimulation of Mammary Lobule-Alveolar Growth.* JOHN P. MIXNER, Dept. of Dairy Husbandry, Univ. Mo., Columbia, Mo.

Ovariectomized virgin female mice were used as assay animals in a study to determine the influence of thyroxine upon the stimulation of mammary lobule-alveolar growth by progesterone and estrone. The criterion of response was the percentage of mice showing positive mammary lobule-alveolar responses after 10 daily subcutaneous injections of the test substances.

Thyroxine at an optimal level increased significantly the percentage of mice which responded with mammary lobule-alveolar growth to minimal doses of progesterone and estrone. There was an increase of 25 per cent in the efficiency of progesterone and estrone in causing such growth.

Thyroidectomy inhibited the ability of the mice to respond to progesterone and estrone with mammary lobule-alveolar growth.

These observations are taken to indicate that the injection of thyroxine or the feeding of thyroprotein during pregnancy might cause greater stimulation of the mammary lobule-alveolar system and thus the potential capacity for milk secretion in subsequent lactation periods. This is in addition to the already recognized stimulating effects of these substances upon the secretion of milk and milk fat during lactation.

P29. The Effect of Thyroxine on Rate of Growth and Efficiency of Weight Increment.† MARVIN KOGER AND C. W. TURNER, Dept. of Dairy Husbandry, Univ. Mo., Columbia, Mo.

Immature virgin female mice were given subcutaneous injections of crystalline thyroxine in dosages varying from 0.015 mg. to 0.04 mg. daily. During an experimental period of 5 weeks, the thyroxine treated mice gained 38 per cent more weight and consumed 25 per cent more feed than suitable controls. The treated animals also stored more nitrogen, while the controls retained more fat.

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* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station Journal Series No. 840.

† Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station Journal Series No. 834.

The thyroxine injected animals gained more in body weight and stored more nitrogen per unit of feed intake than did the controls. This increased mobilization of nitrogen is of particular interest since it may partially explain the mechanism whereby thyroxine treatment increases milk production and rate of growth of animals.

Similarly, the artificial thyroprotein (thyrolactin) was fed and injected into comparable groups of mice for similar periods of time. The growth rate of the experimental animals in both cases exceeded the normal controls.

It is suggested that other animals may respond to proper thyroxine treatment in a manner similar to mice.

P30. Growth and Energy Metabolism of Thyroidectomized Cattle.*

SAMUEL BRODY, Dept. of Dairy Husbandry, Univ. Mo., Columbia, Mo.

The manner in which size and energy metabolism change with age in a thyroidectomized Jersey heifer in contrast to that of a normal control will be illustrated with slides and the practical implications noted. Removing the thyroids at 54 days of age led to reduction in mature body weight by 50 per cent and heat production (metabolism) per square meter by nearly 40 per cent. The animal was completely undeveloped sexually at 3½ years of age. The administration of Reineke's and Turner's "thyrolactin" for three months brought on the first heat period.

P31. The Effect of Thyrolactin on Milk Production, Metabolism, and Growth.†

E. P. REINEKE, Dept. of Dairy Husbandry, Univ. Mo., Columbia, Mo.

Data are presented to show the physiologic effects elicited by the administration of thyrolactin, a preparation of iodinated casein prepared under such conditions as to yield a compound with high thyroidal activity. When administered to tadpoles, either orally or parenterally, thyrolactin stimulates precocious metamorphosis. The percentage decrease in body length of tadpoles is proportional to the logarithm of the thyrolactin dosage. The percentage increase in oxygen consumption of normal, partially-fasted guinea pigs in response to graded doses of thyroxine, given orally, also varies with the logarithm of the dosage. Typical preparations of thyrolactin produce 1 per cent of the response of an equivalent weight of thyroxine when given orally to guinea pigs and 2.7 per cent of the thyroxine response when injected intraperitoneally into frog tadpoles.

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* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station Journal Series No. 832.

† Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station Journal Series No. 839.

Photographs and growth curves are presented to show the effectiveness of thyrolactin in supporting growth and alleviating the symptoms of cretinism of young female goats that were thyroidectomized shortly after birth. Beginning with a dosage of 0.5 gm. daily, the thyrolactin was gradually increased, reaching 2 gm. daily after approximately one year. All animals so treated grew steadily and retained a normal body confirmation. Administration of thyrolactin to an animal in advanced stages of cretinism resulted in rapid improvement. Fed to a three-year-old thyroidectomized Jersey heifer in amounts of 3 to 20 gm. daily, depending upon the potency of the material used, thyrolactin stimulated increases in metabolism of 20 to 30 per cent. The increased metabolism was accompanied by an improvement in appearance and vigor and the initiation of normal estrus cycles. Increases were noted in the milk yield, milk fat percentage and yield of butterfat of lactating cows after feeding thyrolactin for a three-day period.

P32. The Chemical Formation of Highly Active Thyroprotein.* E. P. Reineke, Dept. of Dairy Husbandry, Univ. Mo., Columbia, Mo.

It is demonstrated that the extent of formation of thyroidal substance during and subsequent to the iodination of proteins depends upon the conditions of the reaction. When the iodination is conducted in a medium buffered with sodium bicarbonate, thyroidal activity does not attain the maximum until sufficient bicarbonate has been added to maintain the pH within the range of 6.8 to 8.0. With the concentration of bicarbonate held within the optimal range, the iodine concentration becomes a controlling factor in the formation of thyroidal substance. When the iodine concentration is increased progressively in successive preparations, the thyroidal activity of the resulting product reaches a maximum when sufficient iodine has been combined to substitute two atoms per mole of tyrosine in the protein. Further iodination leads to significant losses in activity. When the concentration of both the iodine and bicarbonate is held constant at the optimal levels, further significant increases in thyroidal activity are obtained by proper control of the iodination and incubation temperature. If both the iodination and incubation are conducted at 38° to 40° C., the reaction involved in the formation of the thyroidal substance soon comes to equilibrium, and no further increase in activity occurs no matter how long the incubation period is extended. The resulting products produce one per cent of the response of thyroxine when assayed orally on guinea pigs or 2.7 of the thyroxine response when injected into frog tadpoles. A pronounced increase in thyroidal activity can be obtained by elevation of the temperature to 60° C. or above either during or subsequent to iodination. The reaction is apparently catalyzed by a metal or combination of metals contained in brass. With the concentration of iodine and bicarbonate at the optimal level, the incubation temperature at 65° to 70° C. and in the presence of a brass stirrer, the thyroidal activity of the resulting product is four per cent of that of thyroxine as assayed orally on guinea pigs or 10.6 per cent as measured by injection into tadpoles. When compared on an equal iodine basis, the effect on tadpoles is equivalent to that of synthetic thyroxine. Crystalline thyroxine has been recovered from a barium hydroxide hydrolysate of iodinated casein, and its identity has been established by its microscopic structure, iodine content, melting point, spectrographic absorption, and tadpole assay.

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* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station Journal Series No. 838.

P33. Methods for Prolactin Assay Including Data on the Anterior Pituitary Prolactin Content of Dairy and Beef Cattle and Female Rabbits in Several Physiological Conditions.*

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Two methods of assay with pigeons—the systemic crop-sac weight and the intradermal local—were studied. With the systemic method, using exclusively 6-week-old, white Carneau pigeons, a more than 2-fold variation in response, over a 1-year period, was noted on groups of 10 or more birds receiving the same amount of the same material (confirming Bates and Riddle). The dose-response relationship of Riddle and Bates was confirmed only for crop-sac stimulation above approximately 2,300 mg. Below this level of response, the slope of the regression line is not as steep as that proposed by the above authors. The influence on the response of route of administration, volume of solution injected, and sex of the birds, was also considered.

By the method of comparing, in the same bird, one crop-sac with the other, after a 4-day intradermal local injection period (method of Reece and Turner) the dose-response curve for extracts and suspensions of anterior pituitary tissue, International Standard prolactin, and purified prolactin, was investigated. It was found by this method that the volume of solution injected, the position of the local injection with respect to succeeding injections, and the presence of muscle extract in purified prolactin solution, were all factors in the response.

Also with this method, acetone dried anterior pituitary powder when administered as a suspension was found to give about the same response as one-fifth as much of the same material injected as an extract. Two samples of bovine anterior pituitary material, one of which was found to be about twice as potent as the other for prolactin by the systemic method, were not distinguishable when tested as suspensions. Yet doubling or halving the amount of a suspension did lead to a noticeable difference in response.

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* This research was supported by an appropriation from Bankhead-Jones funds (Bankhead-Jones Act of June 29, 1935).

The amount of variation in response to the same dosage in a group of birds of the same age and breed was very great. This held for minimum responses as well as for greater. However, under the proper conditions, comparison of the two crop-sacs of the same bird at several levels of response was regarded as satisfactory.

A few assays by the systemic weight method on dairy animals with known history did not indicate more pituitary-contained prolactin than from prime beef steers. Inadequacy of material for an acceptable comparison between dairy and beef cattle necessitated the use of slaughterhouse material. Bulls, cows, and heifers were compared. Assay data from this material by the systemic method on pigeons of the same shipment, as well as assays of extracts by the intradermal local method, does not confirm Reece and Turner in their conclusion that there is more prolactin in the anterior pituitary from dairy breeds of cattle than from comparable beef breeds. Reece and Turner used suspensions of acetone-dried material.

The 2 methods both showed that the sexually mature virgin female contained less prolactin than did the 20-day-pregnant animal. The latter contained about as much as the 8-day-postpartum suckled doe. Pseudopregnant, pseudopregnant spontaneously lactating, and 20-day-pregnant does all contained about the same amount of prolactin. It was also found that the stimulus from suckling brought about perhaps more of a decrease in pituitary prolactin content than was found by Holst and Turner. It is to be noted that we find about five times as much prolactin in the pituitaries of rabbits and cattle in comparison with the International Standard as do the Missouri workers. The approximately 80 per cent of the prolactin not accounted for in their assays, due to the use of suspensions, probably explains most of the differences between their results and ours. The use of the two crop-sacs of the same bird for comparison, as first proposed by Reece and Turner, and which the Missouri group has not always followed, would also probably lead to better agreement with our results.

P34. An Intravenously Active Ovulating Factor in the Juice of Corn and Oat Plants.*

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The investigation of the nature of the rabbit-ovulating factor which, as described by Friedman (1934), occurs in plant juices and is active when injected intravenously into the rabbit, has been continued. This active material can be precipitated from plant juices either by means of benzoic acid or by the addition of sulphuric acid to pH 4. Aqueous solutions (pH 7.4) of these acid precipitates are frequently very toxic and may produce

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* This research was supported by an appropriation from Bankhead-Jones funds (Bankhead-Jones Act of June 29, 1935).

symptoms suggestive of snuffles within a few hours after intravenous administration to a rabbit. Repeated precipitations of the aqueous extracts at pH 4 followed by desiccation with acetone, reduces the toxicity to such an extent that ovulation may be induced with extracts that were lethal initially. Temperatures as high as 85° C. do not destroy the active material in plant juice but are helpful in reducing the toxicity of the extracts.

Corn and oats grown in the greenhouse during the winter months have not yielded active extracts. The variation in potency of field-grown plants has been great (10 to 125 ovulating doses per liter of juice) but these fluctuations have not been correlated either with the stage of development of the plant or the season of the year. Samples of juice were sealed into tinned cans and stored in the freezing room. Preparation of extracts and testing of these frozen juices at frequent intervals have demonstrated that frozen juice will retain its potency for at least 22 months. Furthermore it has been shown that in the late summer and fall there is such a marked decrease in the sensitivity of the rabbit that negative tests were obtained from August to November with doses of extract that gave positive tests in early summer and then again from January to May.

The seasonal variation in the sensitivity of the rabbit is such that a different test animal would be desirable. Preliminary experiments have shown that intravenous or intraperitoneal injection of these plant extracts will induce pseudopregnancy in the adult estrous rat. The usual gonadotrophic hormone assay method of subcutaneous injection into the immature female rat has given negative results in doses that varied from one half to 4 times the effective dose in the rabbit. Feeding of these preparations has not given any indication of being active orally. Further chemical purification and studies on the physiological action of this substance will be necessary before any suggestion can be made for its practical use.

P35. Further Evidence of the Existence and Specificity of an Orally Active Sex Maturity Factor(s) in Plant Juice Preparations.*

E. T. GOMEZ, Div. of Nutrition and Physiology, Bureau of Dairy Industry, Washington, D. C.

The presence of a factor(s) in the juice of the young oat plant that is capable of inducing precocious puberty when administered *per os* to immature rats was reported last year (36th Annual Meeting Amer. Dairy Sci. Assoc. June 1941). The study of this material has been continued. Preparations obtained from the juice of freshly cut young oat and corn plants, that were collected during the growing season of 1941, as well as from the juice of oat plants that were collected in 1940 and frozen and stored for as long as 574 days in a refrigerating room maintained at – 10° C., have brought about vaginal patency (precocious puberty) in rats at 27 to 32 days of age when fed to them beginning at 21 to 22 days of age. In the following report, this plant material will be referred to as SMF (sex maturity factor).

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* This research was supported by an appropriation from Bankhead-Jones funds (Bankhead-Jones Act of June 29, 1935).

Sixteen 21- to 22-day-old rats were fed an SMF preparation from oat or corn juices in amounts each totaling from 200 to 2,000 mg. during the experiment. Fourteen of these rats, which received from 325 to 2,000 mg. of SMF, showed vaginal patency when 27 to 32 days of age. Autopsy of these rats 24 to 48 hours after vaginal patency occurred revealed ovaries weighing from 19 to 31.0 mg. and uteri from 53.0 to 125.0 mg. A like number of litter mate control rats, autopsied simultaneously had ovaries weighing from 15.0 to 18.0 mg., and uteri weighing from 35.0 to 42.0 mg. Gross and histological examinations of reproductive organs revealed varying degrees of development of the follicles or corpora lutea, or both, in the ovaries and there was evident hyperplasia of the endometria. The vaginas of the 2 remaining rats, which received a total of 200 mg. of the preparation of SMF, were closed when autopsied at 32 days of age. The weights of their ovaries and uteri were within the range of those of the controls.

The feeding to each rat of a total of 1,200 μ gm. of vitamin E (alpha-tocopherol) or 400 mg. of vitamin C (Cevitamic Acid, Merck), or 350 mg. of glutathione or of 1,000 mg. of desiccated anterior pituitary tissue of cattle in addition to the basal diet, did not bring about the early vaginal patency of immature rats.

Female pups (foster or otherwise), nursed by a mother fed a potent SMF preparation, beginning at 1 week before or immediately after parturition, showed vaginal patency when 10 to 14 days of age. Autopsy of these pups (9 cases), 24 hours after vaginal patency, revealed ovaries weighing from 7.0 to 10.0 mg., and uteri from 21.0 to 29.0 mg. A like number of control pups (nursed by stock mothers), autopsied simultaneously, had ovaries weighing from 2.0 to 4.0 mg., and uteri from 8.0 to 17.0 mg.

Male pups (7 cases) nursed by SMF fed mothers were autopsied when 15 days of age. The average weight of the gonads was 76.0 mg.; the combined weights of the prostate and seminal vesicles, 25.0 mg.; and seminal vesicles alone, 9.0 mg. The corresponding weights for control pups of the same age were 42.0, 11,8, and 3.4 mg., respectively.

P36. Some Possibilities for the Use of Diethylstilbestrol in Dairy Cattle.*

A. A. Lewis, Dept. of Dairy Husbandry, Univ. Mo., Columbia, Mo.

Diethylstilbestrol has been shown to cause copious and prolonged milk secretion in virgin and dry dairy animals following injection, percutaneous application to the udder, subcutaneously implanted pellets, or oral administration.

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* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station Journal Series No. 829.

Diethylstilbestrol is being used commonly by doctors to relieve the edema, caking, and inflammation of breasts at parturition. Similarly, diethylstilbestrol may be found useful in dairy cattle at this time when the udder is caked, swollen and painful.

There are a number of reports that the use of estrogens is beneficial in some cases of sterility in cattle. Diethylstilbestrol, being a very active estrogen, and cheap, may have some promise in this condition.

Another possibility for the use of diethylstilbestrol in dairy cattle is after abortion in the last half of pregnancy. The mammary glands are prepared for the next lactation by the sixth month of pregnancy. During the last three months what occurs in heifer udders is mostly the beginning of secretion and a swelling of the udder with the accumulation of secretion. Then at calving there is a surge of milk production, due, according to present evidence, to the action of estrogenic hormone in increasing the production of lactogen from the anterior pituitary. In abortion this increase in lactogen may be missing because estrogen has not acted on the pituitary gland. If estrogen is supplied by the injection of diethylstilbestrol it should improve the milk production from such cows.

The possibilities for use of diethylstilbestrol in dairy cattle are great because of its cheapness and great activity. It may be found useful in bringing into production sterile cows which would otherwise be lost to the herd. It may prove beneficial on caked udders at calving, in some cases of sterility, and after abortion. In addition, there are probably other untried possibilities for the use of diethylstilbestrol in dairy cattle. Experimental exploration of these possibilities is indicated.

P37. The Influence of Ascorbic Acid on the Gonadotropic Hormone Content of the Male Rat Pituitary Gland.* R. P. REECE AND E. J. WEATHERBY, N. J. Agr. Expt. Sta., New Brunswick, N. J.

Evidence from several laboratories suggests that ascorbic acid may have an influence on reproductive processes. Because of the established role of gonadotropic hormones in reproduction it was thought worthwhile to determine the effect of ascorbic acid on pituitary gonadotropic hormone content.

Thirty sexually mature male rats were paired on the basis of body weight and age and one of each pair was injected subcutaneously daily for fifteen days with 15 mg. of ascorbic acid. The day after the last injection all of the rats were killed and their pituitary glands weighed. The gonadotropic hormone content of the pituitary glands was estimated by injecting a suspension of each pituitary in eight divided doses over a four-day period into a 24-to-26-day-old rat. The recipients were killed 100 hours after the first injection and the ovaries were weighed.

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* Journal Series Paper of the New Jersey Agricultural Experiment Station, Rutgers University, Department of Dairy Husbandry.

The mean differences of the individual comparisons of the ovarian weights were not significant, thus indicating that the ascorbic acid treatment had no influence on pituitary gonadotropic hormone content.

P38. Vitamin D, the Parathyroid Glands, and Calcium Metabolism.*

I. L. CAMPBELL, Dept. Dairy Husbandry, Univ. Mo., Columbia, Mo.

The parathyroid gland is normally the key regulator of calcium metabolism within the animal body. Its varying activity equates the supply of calcium to the demand for calcium—supply being represented by food calcium absorbed together with mobilizable reserve bone calcium, and demand by the calcium required by the animal for growth, maintenance, and production.

Lactation entails a greatly increased demand for calcium. Data are presented which show that the parathyroids of lactating rabbits are greatly increased in weight over those of normal females, even though these animals are fed good quality alfalfa hay ad lib. and a grain mixture containing one per cent bone meal. This hypertrophy is related to the amount of milk secreted, for when the number of young suckling is reduced to two, there is little increase in parathyroid weight. Heavy lactation involves an increased parathyroid activity.

If the diet is low in calcium, increased parathyroid activity is required for the mobilization of reserve bone calcium. Data are given for normal rabbits and rats to illustrate this point.

Vitamin D promotes the absorption and utilization of calcium. Rats subsisting over several months on a diet low in calcium and in Vitamin D have greatly enlarged parathyroid glands, but if the same ration has Vitamin D added, the glands are found to be significantly smaller, although still somewhat above normal size. Thus a sufficient supply of this vitamin tends to have a parathyroid-sparing action.

P39. Oxygen Uptake and CO_2 Elimination of the Bovine Mammary Gland.

W. E. PETERSEN AND J. C. SHAW, Univ. Minn., St. Paul, Minn.

The oxygen uptake and CO_2 elimination were determined from 157 pairs of simultaneously drawn arterial and venous blood samples from both intact cows and perfusion experiments on active and inactive glands. The average oxygen uptake by the lactating gland of intact cows was 4.78 volumes per cent with 5.59 volumes per cent CO_2 elimination. For perfused active glands the average values for O_2 and CO_2 were 7.52 and 7.37, respectively. For intact dry glands the O_2 uptake and CO_2 elimination was 5.28 and 3.02 while the corresponding values for the perfused dry glands were 6.79 and 7.32.

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* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station Journal Series No. 835.

The average respiratory quotients were: 1.20 for intact lactating; 1.05 for perfused lactating; 0.76 for intact dry; and 1.11 for perfused dry glands. The striking feature about the data is the great variation in O_2 uptake, CO_2 elimination and respiratory quotients. The oxygen uptake for the lactating intact gland varied from 2.7 to 7.0 volumes per cent while the CO_2 elimination in this group varied from 1.51 to 9.27 volumes per cent and the R. Q. from D.625 to 2.26. The variability for intact dry glands was of the same magnitude as was also the case with the perfusion experiments.

No correlation between the glucose uptake and O_2 uptake or CO_2 elimination was found. Nor were there any correlations between the time interval after milking and the drawing of the blood samples. It was also noted that the variations in O_2 consumption and CO_2 elimination were as great in the experiments where there were no blood volume changes as in the cases where there were marked changes in blood volume.

P40. The Enzymatic Hydrolysis of Diacetin by Bovine Mammary Gland Tissue. PHILIP L. KELLY, Ark. Agr. Expt. Sta.

Previous work on this project has indicated an active lipase was present in bovine mammary gland tissue which acted on such substrates as tributyrin, tripalmitin, trilaurin, and ethyl-oleate. While the activity per gram of tissue is not large, the total hydrolysis on the basis of total weight of secretory tissue in the gland would be quite a significant factor to consider in milk fat synthesis.

In the work to be reported in this paper a substrate of smaller molecular weight, diacetin (glycerol diacetate) was used. With tissue previously prepared by drying with acetone and ether there was a great deal more enzymatic activity with this substrate than a comparison with the substrates previously used would indicate. While diacetin as such is not an important compound in milk secretion, the fact that it is acted on so readily by tissue enzymes would indicate that similar compounds which are important in this process might be similarly affected.

P41. The Effect of the Continuous Injection of Pitocin upon Milk and Milk Fat Production. C. B. KNODT AND W. E. PETERSEN, Univ. Minn., St. Paul, Minn.

The injection of the oxytocic principle of the posterior lobe of the pituitary in the form of pitocin has been studied in relation to the ejection of milk in the mammary glands of the bovine. In this experiment an attempt was made to observe the effect of the injection of pitocin following each normal milking upon total milk and milk fat production as well as the percentage of milk fat.

Four cows were utilized in a double reversal type of experiment during a six week period. Two cows were injected following the regular milking and then the "Pitocin Milk" was removed during the first and third fourteen day periods while the other two cows were injected only during the second fourteen day period with the first and third fourteen day periods serving as controls. One milliliter of pitocin (10 units) was used as the standard quantity for all injections. The injections were made intrajugularly immediately after the normal milking and the "Pitocin Milk" removed thereafter.

These cows were in the late stages of lactation and a significantly higher total milk production was noted during the periods of injection as compared to the control periods of non-injection. Three of the cows showed an increase in the percentage of milk fat in the total milk produced during the period of injection while the results on the fourth cow were not as conclusive.

A marked decrease was observed in total milk and milk fat production following the periods of injection. In all but one of the cows there was an increase in the proportion of "Pitocin Milk" with a concomitant decrease in normal milk production as the period of injection progressed. This observed decrease in normal milk may be due to the large amount of pitocin injected and while a smaller amount may produce a similar increase in total production the amount of normal milk might conceivably be increased or at least not so significantly decreased.

It would appear from these experiments that both the amount of milk and milk fat produced may be dependent upon the completeness of the evacuation of the elveoli at the time of milking and that the failure of the cow to completely let down her milk may account for a rapid decline in milk production with the advance of lactation.

P42. The Incidence and Control of Milk Fever.

C. F. MONROE, W. E. KRAUSS, T. S. SUTTON, AND W. D. POUNDEN, Dairy Dept., Ohio Expt. Sta., Wooster, Ohio.

The importance of preventing milk fever was impressed upon us in the maintenance of a herd of mature Jersey cows used in pasture investigations. These cows were bred so that they would calve in late winter and early spring, seasons of the year when the incidence of milk fever is high.

In order to study the effect of the feeding program, the normal dry cow ration of corn, oats, wheat bran, minerals and salt was compared with one containing so-called conditioning feeds composed of oats, wheat bran, linseed oil meal, beet pulp, molasses, minerals and salt. These grain mixtures were compared in two different years, so that all the cows received both mixtures. Out of 34 freshenings, there were 7 cases of milk fever, of which 4 were on the conditioning ration containing molasses. Although these data are too limited for definite conclusions, they do show that milk fever can occur even when the so-called conditioning feeds are used.

Following this, irradiated yeast has been tried, since some work reported by Sjollema indicated favorable results in preventing milk fever by fortifying the ration with vitamin D, dry irradiated yeast in amounts to furnish approximately one million units of vitamin D daily to each cow has been fed for a period of 30 days previous to and one week following freshening. For a control, comparable cows have received the same general care and feedings but without the supplementary yeast. The investigation has been extended to not only include the Pasture Farm Herd, but also the main Experiment Station Herd and that of the Ohio State University.

The data on this work accumulated to this time consist of 75 freshenings of Ayrshire, Guernsey, Holstein and Jersey cows, first parturitions not being included.

The weight of the evidence indicates that the feeding of irradiated yeast reduced the incidence of milk fever. Although there have been cases of milk fever when the yeast was fed, in some of these at least there existed complicating conditions which have nullified a possible benefit from the supplement. On the other hand, a "qualitative" analysis of the data from those cows known to be subject to this trouble suggests that the yeast feeding may have been of some benefit.

In twenty-six freshenings of the milk fever "addicts" the incidence of milk fever was 73 per cent when no yeast was fed and 20 per cent when yeast was fed. The later percentage would be materially reduced if those cases where known complications existed were omitted in the calculations.

P43. The Blood Picture in Normal and Milk Fever Cows.

W. E. Krauss, C. F. Monroe, R. G. Washburn, J. W. Hibbs, T. S. Sutton and N. Van Demark, Dairy Dept., Ohio Expt. Sta., Wooster, Ohio.

In a preliminary trial with a small herd of Jerseys containing several cows with milk fever histories, it was found that when 1,000,000 units of vitamin D as irradiated yeast were fed daily for 3 to 4 weeks before freshening the changes in calcium, phosphorus and vitamin D concentration of the blood were less marked at calving time than when the unsupplemented herd ration was fed. This led to a more detailed study in three herds involving up to the present time 75 calvings of all Jersey, Guersey, Holstein and Ayrshire cows, exclusive of first calf heifers, in these herds.

Half the cows receive 1,000,000 units of vitamin D daily as irradiated yeast for 4 weeks before and one week following freshening. Blood samples are taken four weeks before the due date, within 12 hours of freshening, within 12 hours after freshening, and one week post freshening. If a cow develops milk fever blood samples are taken each day for a week.

These blood samples are analyzed for calcium and phosphorus and, when a cow develops milk fever, for acetone bodies to detect possible complications with acetonemia. Vitamin D is determined on dried pooled blood samples of cows of the same breed freshening within a 4 to 8 weeks' period of each other and on individual samples obtained during attacks of milk fever.

In general blood calcium, phosphorus and vitamin D dropped just before calving, this change being less in yeast-fed animals. During attacks of milk fever blood calcium dropped to as low as 4.0 mg. per 100 cc. and phosphorus to less than 1.0 mg. per 100 cc. Vitamin D in the blood was also materially reduced. Whereas 1.0 gram of dried blood will normally produce 1.0 to 2.0 plus healing in rachitic rats, from 2.0 to 3.0 grams of dried blood obtained from a cow in milk fever were required to obtain the same effect. This, plus the demonstration that vitamin D changes are more marked in Jersey blood than in Holstein blood and that incidence of milk fever was much greater in Jerseys than in Holsteins, suggests strongly that vitamin D plays some role in the prevention of this disease.

P44. Nature of the Material in Mastitic Milk Responsible for the Whiteside Reaction. H. O. Dunn, J. M. Murphy, and O. F. Garrett, N. J. Agr. Expt. Sta., New Brunswick, N. J.

When sodium hydroxide is mixed with milk from mastitic udders a coagulation or gelation occurs, the intensity of which is directly proportional to the degree of infection. The nature of the reaction, known as the Whiteside test, suggests that a protein not present in normal milk is involved in the reaction.

When milk showing a positive Whiteside reaction is allowed to separate by gravity, the cream shows a strongly positive Whiteside reaction whereas the skim milk is negative. Dilution of the gravity cream to the original concentration of the milk results in a positive Whiteside reaction of the same magnitude as the original milk.

When milk showing a positive Whiteside reaction is centrifugally separated, the cream gives a negative Whiteside reaction whereas the skim milk shows a positive Whiteside which is lower in intensity than that of the original milk. Apparently the milk fat is not involved in the typical reaction with NaOH. The fact that the Whiteside reaction was positive in the gravity cream may be explained by assuming that the material involved in the reaction is loosely adsorbed by the fat globules.

Carefully controlled experiments showed that the amount of separator slime obtained from mastitic milk was many times greater than that obtained from milk of mastitis-free cows. Moreover, the slime from mastitis-free cows did not give the typical Whiteside reaction whereas that from the mastitic cows gave an intensely positive reaction. Addition of the slime from mastitic milk to Whiteside-negative milk produced a definite positive Whiteside reaction the intensity of which depended upon the amount of slime added.

A high correlation was found to exist between the intensity of the Whiteside reaction, the number of leucocytes and the amount of separator slime.

A chemical analysis of the slime from mastitic milk showed a protein content of about 68% and a phosphorus content of about 1.35% on the dry weight basis.

Calculations show that cellular material in the slime does not account for all the solid substances present, but when a suspension of equine blood leucocytes was added to non-mastitic milk, which contained no leucocytes, typical Whiteside reactions were obtained the intensity of which corresponded directly to the number of leucocytes added. This is strong evidence that the Whiteside reaction is due, directly or indirectly, to leucocytes.

P45. The Value of Tyrothricin (Gramicidin) in a Herd Mastitis Control Program.

C. S. BRYAN, R. E. HORWOOD, AND C. F. CLARK, Michigan Agr. Expt. Sta., East Lansing, Mich.

Until recently dairymen desiring to eradicate streptococcic mastitis from their herds have been compelled to eliminate all infected cows. At this institution 150 cows, that were infected with mastitis streptococci but had no marked indurative changes of their udder, were treated with 150 milligrams of tyrothricin per quarter. Recoveries were obtained in 93 per cent of these selected cases treated. Sanitary measures must be employed to protect the cows without mastitis, including those recovered upon treatment, from reinfection.

P46. Experiences With Lactovaccine in the Control of Mastitis.

C. F. CLARK, C. S. BRYAN, AND R. E. HORWOOD, Michigan State College, East Lansing, Michigan.

A project was set up to test the value of lactovaccine as a curative agent in the treatment of chronic bovine mastitis, and as a preventive. Two herds were used in the demonstration. The microscopic examination of incubated milk samples was used to determine the presence or absence of infection.

Herd A

Group 1. Twenty-eight cows shedding streptococci in their milk were given repeated injections of lactovaccine. Eight (28%) of the cows so treated apparently recovered, as the microscopic test became negative.

Group 2. Fifty-one cows negative to the microscopic test were given repeated injections of lactovaccine to determine if it would prevent mastitis. Six (11%) of this group later became positive to the microscopic test.

Group 3. Fifty-nine cows negative to the microscopic test were used as controls to Group 2. None of these cows were given lactovaccine. Seven (11%) of this group later became positive to the microscopic test.

Herd B

Group 4. Four cows positive to the microscopic test were given repeated injections of lactovaccine; one cow recovered and became negative to the microscopic test.

Group 5. Forty cows negative to the microscopic test were given repeated injections of lactovaccine. Eighteen (45%) of these cows later became positive to the microscopic test.

Group 6. Seventeen cows negative to the microscopic test were not injected, being used as controls on Group 5. Five (20%) of these cows later became positive to the microscopic test.

Conclusion

Under the conditions of this experiment, lactovaccine appeared to have some value in the treatment of chronic bovine mastitis but did not appear to have value as a preventive measure.

P47. Mastitis and Herd Practices in the College Dairy Herd.

Russell E. Horwood, C. F. Clark, and C. S. Bryan, Michigan State College, East Lansing, Mich.

In 1928 the college herd was moved into the present barn. The herd was culled drastically at that time although no systematic program of testing for infectious mastitis was in effect. A number of young outstanding animals were purchased for replacement. Many of these contracted udder infection at an early age. However, due to their outstanding type and production they were maintained in the herd a number of years. In 1938, 1939, and 1940 due to mastitis, breeding difficulties and age, many of these cows were eliminated. This accounts in part for the rapid drop in the incidence of infection at that time.

Production of Infected vs. Non-infected Quarters.[1] Quarter samples of milk from the college dairy herd were studied from July 1, 1933, to May 30, 1934. The samples were from cows with no udder infection and cows having one, two, three or four infected quarters.

In 36 non-infected cows the fore quarters produced 44.9% of the milk and 43.6% of the butterfat.

In 14 cows having opposite infected and non-infected forequarters there was a reduction of 16.4% milk and 16.8% fat in the infected quarters. A similar study on rear quarters with 9 cows showed a 32.4% reduction of milk and 32.2% reduction of fat in the infected quarters.

Methods of Testing. Monthly samples of milk from each quarter were taken and tested for infectious mastitis beginning November 1932 and continuing to February 1937. The following methods were used in detecting infectious mastitis: microscopic test, chloride content, physical examination of the udder, leucocyte content, thybromol test, physical examination of milk and culture of milk and blood agar.[2]

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[1] Unpublished data gathered by George W. Taylor.

The results of the above work lead to the adoption of the microscopic system of testing. Quarter samples from each cow in the herd were tested from June 1937 to June 1938. Composite samples were then run on the negative cows and quarter samples continued on the positive cows. Since October 1941 all quarter samples have been discontinued.

Hand vs. Machine Milking. Starting June 1937 alternate first calving two year olds were placed on hand and machine milking. In addition a number of older negative cows were carried on machine milking. This was continued through November 1940. During this time an average of 15 cows were milked by hand and 25 by machine. Six hand-milked cows and six machine-milked cows became positive to infectious mastitis. Individual machines were marked for use on only negative or infected cows. No success in controlling the spread of disease resulted until this plan was adopted.

Short Wave Diathermy. During the summer of 1939, three cows with infected udders were given 50 one-hour daily treatments with short wave diathermy. Three infected cows were also observed as controls.

Examinations of samples of milk taken at weekly intervals were made from the six cows using the Hotis and Modified Hotis test at 24-, 30-, and 48-hour intervals. We also used the thybromal test, microscopic test and chloride count. A physical examination of the udder was made. No significant variation was found in the milk or udder of either the treated or untreated cows.

Lacto Vaccine Treatment. Starting in May 1939 lacto-vaccine was used to treat twenty-six different cows. Of this number eight showed complete recovery.

Tyrothricin (Gramicidin) Treatment. Tyrothricin has been used to treat nine cows in the herd. Eight of the cows became negative to mastitis. The ninth cow was negative on one test. However, this was a continuous problem cow and died soon after the last test from systemic acute mastitis. Two others remained negative for six months and then developed systemic acute mastitis. One of these died and the other lost the functional use of her udder and was slaughtered.

Summary. At the start of this program in 1932 there were 56 milking cows in the herd. Fifty per cent of these had infectious mastitis. In May 1942 the herd contained 46 milking cows. It passed a 100 per cent clean test and has done so six of the past seven months. This was accomplished by culling the infected cows, preventing the spread of the organisms, and by recovery by treatment of 17 mastitis-infected animals as mentioned previously. There were udder disturbances of a non-infectious nature during this period.

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[2] Results published in Journal of Milk Technology, vol. 2, No. 1, pp. 32–40. 1939.

MANUFACTURING SECTION

M1. The Effect of Acidity and Temperature on the Growth of Oospora Lactis Cultures.* E. R. Garrison, Dept. of Dairy Husbandry, Univ. Mo., Columbia, Mo.

The effect of the reaction of the medium and incubation temperature on the growth of 15 selected cultures of *Oospora lactis* recently isolated from sour cream was determined by measuring the diameter of giant colonies produced on potato dextrose agar plates after seven days incubation. Individual flasks of sterilized medium at a temperature of 113° F. were adjusted to a pH of 9.0, 8.0, 7.0, 6.0, 5.0, 4.0, 3.5, 3.0, or 2.5, as determined by the glass electrode, by the addition of sterile 20 per cent tartaric acid or N/1 NaOH. After standing two hours at 113° F. the reaction was again adjusted to the specified pH (± 0.05) at a temperature of 104° F. The medium was then distributed among Petri dishes in 25-ml. portions and when the agar had solidified the center of each plate was inoculated with a platinum needle that had been dipped into a water suspension of a mold culture. Three plates of the medium from each flask were inoculated with each culture. The diameter of the colonies was measured after seven days incubation at 80° F. The average diameter of the three colonies expressed in mm. denoted the amount of growth of the culture at the particular pH concerned.

The pH curve of growth was very similar for all cultures. The diameter of the colonies of each culture was essentially the same at pH 8.0, 7.0, 6.0 and 5.0; a marked decrease in growth occurred at pH 9.0 and 4.0, while growth was slight at pH 3.0 and negative at pH 2.5. The colonies formed at pH 3.5 were only one-half to one-third as large as those produced at pH 4.0. When the cultures were grown on potato dextrose agar acidified to pH 3.5 with lactic acid the colonies were regularly larger than those produced on this medium acidified to pH 3.5 with tartaric acid; smaller colonies were produced in similar trials in which sulphuric acid was used.

The comparative rate of growth of these cultures in cream of different acidities was also investigated. Four flasks of sweet cream contained 30 per cent fat were pasteurized at 160° F. for 30 minutes, then cooled and treated as follows: (1) not treated, (2) acidified to 0.40 per cent (pH 4.90) by the addition of sterile lactic acid, (3) fermented with *S. lactis* to an acidity of 0.55 per cent (pH 4.50), and (4) fermented with *L. bulgaricus* to an acidity of 1.40 per cent (pH 3.59). Three hundred and fifty grams of each cream sample were weighed into sterile one quart fruit jars and the lids loosely replaced. One jar of each of the four samples was inoculated with 0.1 ml. of a water suspension of a mold culture then plated at once to determine the number of mold spores added. The samples were held at 70°

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F. for eight days and stirred daily with a sterile glass rod. After four days and again after eight days the mold plate count, the mold mycelia content and the per cent acidity of the samples were determined. Essentially the same amount of mold growth occurred in the untreated, acidified, and *S. lactis* ripened cream samples, while a slight reduction in mold growth occurred in the *L. bulgaricus* ripened cream with some cultures but not with others.

The temperature curve of growth of the 15 cultures was studied by measuring the diameter of the colonies produced on potato dextrose agar (pH 5.0) during seven days incubation at temperatures of 40, 45, 50, 60, 70, 80, 85, 90, 95, 100, and 105° F. None of the cultures produced visible colonies during seven days at 40° F. but growth was usually evident after 10 days. The diameter of the colonies increased as the temperature was raised from 45 to 80° F. and decreased sharply at a temperature of 95° F. The optimum temperature for most cultures was near 85° F. Two cultures made essentially the same growth at 80° as at 85° F., while four cultures produced colonies of approximately the same size at 85° and 90° F. Five cultures made a slight growth at 100° F. but none of the cultures grew at 105° F.

M2. Various Treatments Which Affect the Growth of Mold Mycelia in Cream and Resultant Butter.*

J. E. EDMONDSON AND W. H. E. REID, Univ. Mo., Columbia, Mo.

This investigation is concerned with a study of the various factors affecting the development of mold in cream and butter. The following factors have been studied: time or storage period, variable storage temperatures, stirring and non-stirring of cream samples, layering and non-layering of cream samples, covered and non-covered cream samples, variable butterfat content of cream, and the comparison of wet and dry refrigeration of cream on the farm.

Storage temperature was found important to the growth of mold mycelia in cream since it multiplies rapidly at the higher temperatures due to the characteristic ability of this mold to tolerate acid. The time factor is also important; however, mold growth can be controlled by the application of a four-day delivery system to such an extent that temperature is not entirely a controlling factor.

In the study of creams of variable butterfat content, the data shows definite correlation between the rate of mold growth and the percentage of butterfat. As the butterfat of the cream was increased the mold growth diminished.

A comparison of the stirred and non-stirred samples of cream showed that the mold growth was more apparent in the stirred samples; however, in the finished butter the non-stirred samples had the highest mold count.

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The covered and non-covered samples of cream show that the covered samples have a lower mold count but that the sealing of the container covers resulted in the development of stale, musty and absorbed flavors and aroma. The absorbed flavors were transmitted by the resultant butter, thereby lowering its quality.

The layered and non-layered samples of cream gave results which definitely favor the practice of the layering of cream. The layered samples give a much lower mold count in cream and resultant butter, and may be due to the control of the air supply which is required by the mold.

Studies of the comparison of dry and wet refrigeration are still in progress.

M3. The Development of a Positive Phosphatase Test on Refrigerated Pasteurized Cream. F. W. Barber and W. C. Frazier, Dept. Agr. Bact., Univ. Wis., Madison, Wis.

Vat pasteurized cream, which gave a negative phosphatase test immediately after pasteurization, developed a positive reaction to the test after being held at 4° to 10° C. for 24 to 48 hours. Gram positive, spore-forming bacilli were isolated from each lot of cream and grew in pasteurized cream, sterilized cream and sterilized skim milk with the production of phosphatase. There was no evidence of the production of phenol or phenol-like compounds. The bacilli isolated vary somewhat in their morphological characteristics, but apparently all belong in the mesentericus group.

The phosphatase produced by these organisms appeared early in the medium and washing of the cells resulted in positive tests on the wash water.

These organisms produced phosphatase more rapidly and with fewer numbers present in pasteurized cream than in sterilized cream. Microscopic counts were one to two million/ml. in pasteurized cream but in sterilized cream, before phosphatase activity developed, counts were anywhere from 48 to 434 million/ml.

Experiments indicated that phosphatase was not protected from destruction during the pasteurization process and later liberated in sufficient amounts to give a positive test.

Present evidence indicates that the phosphatase production by these organisms is a contributing factor in the change in reaction of the phosphatase test from negative to positive on refrigerated pasteurized cream.

M4. The Keeping Quality of Cream Pasteurized at 165° F. for 30 Minutes, Variously Treated, and Stored at 0° F. E. S. Guthrie, Cornell Univ., Ithaca, N. Y.

Twelve lots of 12 samples each of cream pasteurized at 165° F. for 30 minutes were stored at 0° F. in sealed three-quart-tin cans. The factors studied were: homogenization, deaeration, addition of ascorbic acid, and addition of isoascorbic acid.

Flavor examinations were made after storage of 7 months, and again after 19 months. The results of the quality scores showed only slight differences.

M5. The Keeping Quality of Unsalted Butter Made from Sweet Cream Pasteurized at 165° F. for Thirty Minutes and Stored at 0° F. and 32° F. C. N. STARK, E. S. GUTHRIE, AND J. J. R. CAMPBELL, Dept. of Dairy Indus., Cornell Univ., Ithaca, N. Y.

Previous reports from this laboratory have shown the superior keeping quality of unsalted butter made from sweet cream pasteurized at 165° F. for thirty minutes. Many reports in the literature of the dairy industry show the importance of refrigeration of ordinary commercial butter at temperatures around zero degrees F.

Our laboratory findings indicated to us that butter might be satisfactorily held at appreciably higher temperatures if the more important butter spoilage factors were properly controlled.

Experiments have been conducted to obtain data on this point. Unsalted butter made from sweet cream pasteurized at 165° F. for thirty minutes has been held at 0° F. and 32° F. and judged for quality at various intervals. For the first six months of storage the butter held at 32° F. is of excellent quality as is the butter stored at zero ° F. The butters stored at both temperatures scored 93 at the end of six months' storage. The practical value of such results is obvious. These experiments are being continued.

M6. Some Observations Concerning the Ascorbic Acid Content of Evaporated Milk. D. V. JOSEPHSON AND F. J. DOAN, Pa. Agr. Expt. Sta., State College, Pa.

Four series of evaporated milk samples were obtained over a period of two years from twenty-seven different manufacturing plants in various parts of the United States. Samples were reconstituted to a fluid basis and analyzed soon after receipt and again after two and four month intervals of storage at room temperatures.

The greatest loss in ascorbic acid content apparently took place during processing and immediately thereafter. Assuming an average figure of 20 mg. of ascorbic acid per liter for the milk before processing, it was found that 50 to 95 per cent (with an average of 76.6 per cent) of the vitamin was lost during this period. In the following two months of storage the reduced ascorbic acid content was depleted an average of about 1 mg. per liter of reconstituted milk, or 5 per cent more, while during the second two month period very little, if any, further reduction was noted.

Dehydroascorbic acid analyses by hydrogen sulphide and bacterial reduction techniques, indicated that very little of this form of the vitamin was present to begin with or developed during storage.

The copper content of the evaporated milk samples analyzed in this study varied from .36 to 2.2 p.p.m. on a reconstituted basis, with an average of .78 p.p.m. Of the twenty-seven plants submitting samples, twenty, or 74 per cent were using copper vacuum pans. The seven others employed stainless steel pans, but only three of these were using stainless or non-copper auxiliary equipment.

Although the copper content of the evaporated milk varied widely, there was no correlation between the copper content and the amount of reduced or dehydroascorbic acid present, either before or after the four months storage period.

Only five of the twenty-seven plants submitting samples were not irradiating the milk, but from the limited number of comparisons available, irradiation appeared to have no effect on the ascorbic acid content of the finished evaporated milk.

The stability of ascorbic acid appeared to be somewhat greater in evaporated milk made during the spring and summer, but there was no apparent correlation between the kind or amount of stabilizing salt used and the ascorbic acid content.

The pH of the evaporated milk samples as received ranged from 6.1 to 6.35. During storage a further and progressive decrease was noted which seemed to be less marked in the case of milk made in the pasture season.

"Spangling," or discoloration characteristic of the inner surface of evaporated milk cans after processing and storage, was found to become progressively worse on holding, but cans containing milk of higher copper content generally exhibited less "spangling" than those containing milk of low copper content.

M7. Control and Verification of Vitamin D in Milk. M. J. DORCAS, National Carbon Co., Inc., Chicago, Ill.

Recognition is given to the part of the control official and his supervision of methods in establishing and maintaining the high quality of milk that is now generally available in this country. One of the best examples of this system is the control of the pasteurizing process. Vitamin D milks prepared by the irradiation process are now capable of an analogous system of control through the use of recording type meters which scientific research has shown to give an accurate guide to the potency produced and the records of which fit in well with the established methods of control now in use by the health officials.

Supplementing this constant daily check giving a record of the potency of each pound of milk handled the Vitamin D product is further checked by periodic assays or analysis of samples of the milk. The method of analysis used, that of bio-assay with rats, is satisfactory with respect to accuracy but is somewhat expensive and requires a period of several weeks. It is probable that a rapid chemical analysis of milk for Vitamin D may become available because of progress in Vitamin D assays by chromatographic methods which are briefly described.

M8. A Voltammetric Method for Measuring the Concentration of Dissolved Oxygen in Dairy Products. G. H. HARTMAN AND O. F. GARRETT, N. J. Agr. Expt. Sta., New Brunswick, N. J.

The dropping mercury electrode method of measuring the concentration of dissolved oxygen has been applied successfully to milk. The apparatus used included an electrode assembly consisting of a capillary mercury electrode and a saturated calomel half cell, a sensitive reflecting mirror galvanometer, a photoelectric cell and amplifier, and a thread and drum recorder. The recorder marks the galvanometer deflection at minute intervals.

The relationship between the concentration of dissolved oxygen in milk and the magnitude of the galvanometer deflections was found to be linear. The concentration of oxygen, therefore, is proportional to the galvanometer deflection. The determination in milk may be made at a potential ranging from 0.8 to 1.2 volts.

The method actually determines the concentration of dissolved oxygen in the aqueous phase of milk. The comparatively slight variations in the solids content of normal milks, however, do not introduce significant errors.

A probable error of ± .03 and a standard deviation of 0.18 of the mean oxygen in 21 samples of air-saturated whole milk of varying solids composition show that the method is reliable.

The method and the instrument permit a continuous recording of the oxygen content of a sample. This feature should aid greatly in studying various oxidation reactions which occur in milk.

M9. Studies of the Mechanisms of Oxidized Flavor. W. CARSON BROWN AND FLOYD C. OLSON, W. Va. Agr. Expt. Sta., Morgantown, W. Va.

An attempt has been made to obtain an insight into the mechanism for the production of oxidized flavor in milk. Washed cream from which practically everything had been removed but the fat globules and their membranes was found to be a suitable medium for study. The presence or absence of oxidized flavor was determined by taste.

It was found that washed cream from susceptible milk did not develop an oxidized flavor when contaminated with copper, but that the same cream with from 20 to 200 mg. of ascorbic acid per liter developed a strong oxidized flavor both with and without added copper. Larger amounts of ascorbic acid were found to prevent the development of the flavor. Non-susceptible milk, produced on pasture, likewise, was found to develop an oxidized flavor under similar conditions. Added glutathione, in the presence of copper, was found to produce an oxidized flavor. However, no evidence was obtained that thiamine, riboflavin, pyridoxine, or cysteine, were involved in the production of oxidized flavor.

Not only did reduced ascorbic acid produce oxidized flavor in washed cream but dehydroascorbic acid likewise produced it. Evidence from these experiments indicates that the two steps of oxidation of ascorbic acid are first to dehydroascorbic acid and then to oxalic acid and threonic acid. Both reactions probably reduce copper from the cupric to the cuprous form. The cuprous copper being very unstable is then oxidized by molecular oxygen to the cupric form with the production of hydrogen peroxide. The hydrogen peroxide then oxidizes the phospholipides of the fat globule membrane producing decomposition products that cause oxidized flavor.

A similar production of hydrogen peroxide by the oxidation of glutathione from the sulfhydryl to the disulfide form is indicated in the literature. Hydrogen peroxide in the presence of copper was shown to develop an oxidized flavor in washed cream. However, it is believed that one of the main mechanisms of oxidized flavor production in milk is found in the ascorbic acid.

M10. Relation of Dissolved Oxygen to Certain Oxidation Reactions in Milk.

G. H. HARTMAN AND O. F. GARRETT, N. J. Agr. Expt. Sta., New Brunswick, N. J.

The application of the dropping mercury electrode method for measuring the concentration of dissolved oxygen in milk has made possible a study of the chemical oxidation reactions which occur in milk in a more exact manner than was formerly possible.

The rate of oxygen consumption by the following oxidations in milk has been studied: 1. Oxidation of ascorbic acid with and without copper contamination; 2. Development of oxidized flavor.

The ratio of oxygen consumed to the amount of ascorbic acid oxidized is about the same in uncontaminated and copper-contaminated milk. The ratio varies in different milks and ranges from 1 molecule of ascorbic acid per 1.2 to 1.6 atoms of oxygen.

When the concentration of oxygen in copper-contaminated milk is plotted against time, the curve shows a rapid decrease in oxygen so long as ascorbic acid is present. When all the titratable ascorbic acid has been oxidized the oxygen concentration remains constant for a period of time the length of which varies with different milks. At the point at which the first perceptible oxidized flavor appears a decrease in oxygen concentration occurs. There follows a slow but progressive decrease in oxygen concentration as the tallowy flavor becomes more intense. The amount of oxygen needed to produce a barely perceptible oxidized flavor is small but is considerable for the development of an intense flavor. In a sample of fresh pasteurized milk which contained 19.45 mgs. of ascorbic acid per liter and to which copper was added, the oxygen concentration decreased from 8.58 p.p.m. at the onset of oxidized flavor to 6.85 p.p.m. when the flavor reached an intensity of T^{+++}. The corresponding decrease in oxygen in the same sample of milk to which was added approximately 25 mgs. of ascorbic acid per liter was from 5.68 p.p.m. to 3.81 p.p.m.

M11. The Role of the Oxidase Producing Bacteria in the Development of Oxidized Flavor in Milk. J. Frank Cone and C. J. Babcock, Bureau of Dairy Industry, U.S.D.A., Washington, D. C.

A study was made to determine the ability of the oxidase-producing bacteria to cause oxidized flavor in pasteurized milk. Nine strains of gram-negative rods were used in the study, each strain capable of giving a positive reaction when agar plates on which they were grown were flooded with a 0.5 per cent aqueous solution of p-aminodimethylanaline monohydrochloride. They included *Pseudomonas putrefaciens, Ps. fragi,* and seven unidentified strains which were isolated from milk. In addition an oxidase-negative strain of *Achromobacter lipolyticum* was used in some of the experiments.

Milk shown by previous studies to be susceptible to the development of oxidized flavor either spontaneously or upon the addition of not more than 0.1 p.p.m. of copper, was pasteurized in flasks at 63° C. for 30 minutes. Different lots of this milk were inoculated with the experimental organisms, either single strains or combinations of strains. The amount of inoculation was varied to give counts in the freshly inoculated milk ranging from a few hundred to several million per ml. Each freshly inoculated lot was distributed in half-pint bottles, one for examination immediately and two for examination after 2 and 4 days' storage respectively at 4–6° C. The examination consisted in tasting for evidence of oxidized flavor and plating on standard TGEM agar to determine total counts (at 20° C. incubation) and numbers of oxidase positive organisms. Suitable controls were run without inoculation, with and without the addition of 0.05–0.1 p.p.m. of copper.

No inoculated sample became oxidized except when the corresponding uninoculated control also became oxidized and the intensity of the flavor in those inoculated samples that did become oxidized was never greater than in the control. When inoculations of several million per ml. were made there was definite evidence that the organisms protected the milk against the development of oxidized flavor.

M12. Bacteriological Studies on Creamery Water Supplies. R. T. Corley and B. W. Hammer, Iowa Agr. Expt. Sta., Ames, Iowa.

Examinations of 436 samples of unchlorinated water from 70 butter plants, using the common bacteriological tests and a few additional ones, indicated that some plant supplies regularly were satisfactory, some regularly were unsatisfactory, while others varied in quality from one examination to the next. Certain supplies that would have been considered acceptable from a public health standpoint were not suitable for use in butter manufacture. In some plants satisfactory well water was heavily contaminated in storage tanks. Some city water supplies were unsatisfactory for butter manufacturing purposes.

When water known to contain coliform organisms was used to wash experimental butter, the organisms regularly were found in unsalted butter and sometimes were found in salted butter. Commercial butter from plants using water that commonly contained coliform organisms regularly contained the organisms when unsalted and sometimes contained them when salted. As coliform organisms become more numerous in water, total bacterial counts on the water tended to be higher.

T.G.E.M. agar counts after 96 hours at 21° C. were higher than the counts on this medium after 48 hours at 37° C. The medium gave higher counts than nutrient agar after 24 to 48 hours at 37° C.

Ps. putrefaciens was found in a considerable number of water samples, while typical fluorescent organisms were found in a still larger number. Some *Pseudomonas* species known to produce undesirable flavors in butter were isolated from some water supplies. These included *Ps. fragi, Ps. graveolens* and *Ps. mephitica.*

Some water samples having high bacterial counts did not cause flavor deterioration when used to wash experimental unsalted butter, but the tendency was for samples showing high bacterial counts to be more generally associated with serious deterioration in butter than samples having low counts.

M13. Sensory Adaptation as a Factor in the Judging of Dairy Products for Flavor.

S. T. COULTER, Dairy Dept., Univ. Minn., St. Paul, Minn.

It is a common experience to note that an odor which is almost overpowering when first smelled soon becomes imperceptible. This adaptation of the olfactory receptors is a definite factor in the reliability of the judgment passed on the flavor of dairy products.

Trials with seventeen individual judges showed a fairly rapid, although variable, rate of adaptation to the odor of coumarin. Most judges could readily detect initially the odor of coumarin in a 0.005 per cent aqueous solution. Following one sniff of a 0.3 per cent solution, many did not detect the odor in a 0.012 per cent solution. After as many as 3 sniffs of a 0.3 per cent solution, none detected the odor of coumarin in the 0.005 per cent solution. All judges readily detected coumarin in ice cream flavored at the rate of 4 ounces per 5 gallons of mix with a standard pure vanilla extract to which 2 grams of coumarin per gallon had been added. If, however, a duplicate sample of the same ice cream was preceded by two samples flavored with the same amount of an extract containing 60 grams of coumarin per gallon, the sample was quite uniformly picked as one flavored with pure vanilla.

Similar results were secured in the judging of butter and cream flavored with garlic.

M14. A Quick, Colorimetric Method for Estimating the Quality of Butter.

E. S. GUTHRIE AND GEORGES KNAYSI, Cornell Univ., Ithaca, N. Y.

A simple method of estimating the quality of butter is described. It consists in dissolving 1 ml. of the melted milkfat in chemically pure xylol saturated with the base of neutral red, and comparing the color with standards containing known quantities of oleic acid. The preparation of the base of neutral red is described.

The test, which is a measure of the degree of the hydrolysis of the milkfat, is found to be of value in quickly detecting bad samples, and the majority of fair or good samples of butter, and in adding precision to the judgment of the expert. Over 100 samples of butters of various qualities were examined.

M16. Forewarming Temperature of Plain Condensed Skimmilk and Properties of the Resulting Ice Cream.

JACK B. CLINCH AND J. H. ERB, Dept. of Dairy Technol., Ohio State Univ., Columbus, Ohio.

The variations in properties of ice cream caused by using plain skim condensed milks subjected to different forewarming temperatures were studied.

Raw skimmilk was divided in three lots and forewarmed in a jacketed stainless steel hot well for twenty minutes at 160° F., 180° F. and 200° F. Each lot of milk was then condensed to 40% solids in a 16-inch stainless steel vacuum pan. Relative viscosities were determined upon each sample at several concentrations between 30% and 40% total solids. In some trials the milk was condensed to 30% solids content.

The viscosity results indicated that the higher the temperature of forewarming the greater was the viscosity of the milk when condensed to 40% solids. There was a marked difference in the viscosity of 40% condensed milk preheated at 160° F. and that preheated at 200° F. The viscosity of that preheated at 180° F. was midway between the other two.

When the condensed milks were incorporated into ice cream mixes, as the chief source of serum solids, differences could be detected in the resulting ice cream depending upon the heat treatment of the skimmilk. In general, the preheating temperature of 200° F. imparted to ice cream a more resistant, or "chewy" body, and produced a product that melted smoother and slightly more rapidly. This was especially significant when the condensed milk was condensed to 40% solids. The results indicated that lactose crystallization developed more rapidly in the ice cream containing the condensed milk forewarmed at 200° F. Little difference in flavor was noted in the mixes with the various heat-treated condensed milks.

M17. Relation of Different Mix Compositions and Methods of Processing to the Texture, Structure and Stability of Ice Cream.* C. W. Decker and W. H. E. Reid, Mo. Agr. Expt. Sta., Columbia, Mo.

Based upon a standard mix containing 1 per cent of sucrose, a partial replacement of sucrose with dextrose was made as follows: 15 per cent sucrose (0 replacement), 1¼ per cent sucrose, 3¾ per cent dextrose (25 per cent replacement); 10 per cent sucrose, 5 per cent dextrose (33⅓ per cent replacement). Mixes of variable compositions of 11 per cent serum solids—12 per cent butterfat, 11 per cent serum solids—14 per cent butterfat, 13 per cent serum solids—12 per cent butterfat, and 13 per cent serum solids—14 per cent butterfat were also studied.

Drawing temperatures of 24° F. in the batch freezer, and 24° F., 23° F., 22° F. in the continuous freezer were used to determine their relation to the physical properties of ice creams.

Thin sections of ice cream of 10 to 15 microns in thickness were prepared and immersed in an immersion media of ethyl acetate and photomicrographs taken. Fifty or more measurements were made from each photomicrograph to determine the ice crystal, also air cell, size, distance between ice crystals and distance between air cells. An average was determined from the fifty or more measurements and an analysis of variance made statistically with the data.

A table of recommended drawing temperatures based upon observations of the ice cream when drawn from the freezer and ice crystal sizes from microscopic studies was drawn up for the mix compositions studied.

It was found that as the drawing temperature was decreased from 24 degrees Fahrenheit to 22 degrees Fahrenheit the size of the ice crystal decreased markedly. This resulted in an ice cream with a smoother, finer texture and structure.

Variations in the composition also had an important influence upon the texture and structure of ice cream. The difference between a 25 per cent and 33⅓ per cent replacement of sucrose with dextrose was not sufficient to give consistent distinctions of the ice crystal size of the resultant ice creams. An increase in the serum solids content from 11 per cent to 13 per cent did not have as important an effect on the ice crystal size as did an increase of butterfat from 12 per cent to 14 per cent. The increase of butterfat content resulted in a finer ice crystal and a smoother texture in the ice cream.

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The influence of variations in composition upon the meltdown properties of the ice cream was quite apparent. An increase in butterfat gave a meltdown which was characterized by sluffing off, and a coarse external appearance. An increase in the serum solids content gave a desirable, smooth meltdown characterized by a melting of the ice cream to the original mix. The variations in drawing temperatures did not have any visible influence upon the meltdown appearance of the ice creams.

M18. The Gases Evolved by Cheddar and Limburger Cheese.* F. L. DORN AND A. C. DAHLBERG, New York (Geneva) Agr. Expt. Sta., Geneva, N. Y.

The ripening of natural cheese in tight tin cans made it convenient to study the volume and composition of the gas evolved during the curing process.

The volume of gas produced by cheddar cheese was more uniform and much less when made from pasteurized milk, especially when cured at 40° F. The evolution of gas was most rapid during the first month of curing but it did not cease completely even after one year.

The oxygen in atmospheric packed cheese disappeared in one week at 60° F. but 7 weeks were required at 40° F. The gas evolved from the cheese was nearly pure carbon dioxide with traces of hydrogen. The loss in weight of the cheese due to the carbon dioxide evolved varied from .13 to .20% for raw milk cheese and .04 to .09% for pasteurized milk cheese. The milk was of market milk quality. The gas evolved per pound of cheese made from raw milk varied from 307 to 491 ml. in 44 weeks at 50° F. and 254 to 296 ml. at 40° F. For pasteurized milk cheese the volume of gas was 121 to 197 ml. in 44 weeks at 50° F. and 86 to 87 cc. at 40° F.

Gas formation in commercial raw milk Limburger cheese was much greater than in cheddar cheese. When the Limburger cheese was partially cured before canning the gas was 90 to 95% carbon dioxide and 5 to 10% hydrogen. Fresh Limburger curd produced gas that was 95 to 97% carbon dioxide. The partially ripened Limburger cheese when canned produced from .8 to 3 liters of gas per pound of cheese in 18 weeks at 50° F. and .3 to 1.3 liters at 40° F. This volume of gas represented a loss of .6 to 1.2% of the weight of the Limburger cheese.

M19. The Preparation of Crystalline Rennin. C. L. HANKINSON, The Carnation Research Laboratories, Milwaukee, Wis.

Previous progress in the purification of rennin from commercial rennet extract (JOUR. DAIRY SCI., **25**: 277 (1942)) resulted in the isolation of a highly purified, though not crystalline, enzyme concentrate. Continued study has resulted in actual crystallization of the enzyme.

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* This investigation was supported by Bankhead-Jones funds.

The principles of purification employed were isoelectric precipitation from salt solutions, centrifuging and dialysis. Precipitation from saturated sodium chloride solutions at pH 5.0 rather than from 16.7 per cent sodium chloride solutions at pH 4.5 had the following advantages: (1) a higher potency product, (2) less total losses of active material, and (3) less difficulty with amorphous material in the subsequent crystallization procedure.

The highly purified rennin concentrate was dialyzed, diluted to a solids concentration of 0.05 per cent, filtered and the pH adjusted slowly to 4.5 with N/10 HCl. Rennin crystallized out as long white needles and after standing several hours at room temperature the white crystalline material was centrifuged off. Recrystallization may be effected by repeating the crystallization procedure but this has been found to be unnecessary because of losses of active material with no further increase in activity per unit weight.

Care should be taken in the crystallization procedure to prevent precipitation of amorphous material which may result from the following:

(1) Too rapid addition of acid.
(2) Solids content greater than 0.05%.
(3) Insufficient purification prior to crystallization.
(4) Room temperature greater than 20 to 25° C.

Some of the chemical properties of pure renin will be reported.

M20. The Use of Rennet Paste in Romano-Type Cheese.

C. A. PHILLIPS, G. A. RICHARDSON, AND N. P. TARASSUK, Univ. Calif., Davis, Calif.

Domestic rennet paste from three different sources, and one sample of imported Italian paste, were compared with liquid rennet in the manufacture of Romano-type cheese.

Since April 1941, fifty-four cheese have been made, the average cured weight of each being approximately 20 pounds. Holstein milk, partially skimmed, was used, a stirred curd produced, the cheese dry or moist salted, and cured at 55° F. A mixture of cottonseed oil and black pepper was applied to the surface of the cheese at approximately one month intervals during the curing process.

The cheese was analyzed for milk fat, total solids, and salt. Determinations for pH value were made during the curing process. These analyses conformed very closely to that of one sample of imported Italian Romano cheese which was as follows: total solids, 63.03 per cent; milk fat, 39.2 per cent in the dry matter; salt, 5.87 per cent; pH value, 5.13.

The cheese made with liquid rennet was totally lacking in typical Romano flavor. All samples of rennet paste used produced cheese with a desirable piquant flavor, similar to that in the Romano cheese imported from Italy. Homogenization of the milk or the addition of steapsin caused the development of excess piquancy when rennet paste was used.

The voltaile acid production in the cheese made by the various procedures was determined using an extraction method.[1] The results indicate that the increased piquancy induced by rennet paste over liquid rennet is due to a greater production of volatile acids rather than to a specific lipolysis. There is reason to believe that homogenization of the milk as well as the addition of steapsin produces not only a greatly increased lipolysis, but also an accelerated liberation of the volatile insoluble acids.

M21. Studies Relating to the Canning of Pasteurized Milk Cheese.

A. C. DAHLBERG AND J. C. MARQUARDT, N. Y. Agr. Expt. Sta., Geneva, N. Y.

The principal problem to be overcome in the successful canning of natural cheese is the disposition of the evolved gas. This has been successfully done with the vent can but not with the less expensive conventional tin can used in these experiments.

There are several important factors that are involved in curing cheese in the ordinary tin can. The cheese should be made from cheese-milk of good quality. The milk should be pasteurized to destroy most of the gas-producing bacteria. The cheese should be well made without excessive moisture and packed with 20 to 25 inches of vacuum. The vacuum aids by serving as a buffer area to take up some of the gas. The rate of gas formation is least with cold curing. Finally, to secure most permanent assurance against gas pressure, a small amount of calcium or magnesium hydroxide may be placed in the cans to combine with the carbon dioxide evolved during ripening.

Natural cheese may be vacuum packed and ripened in the cans for 4 to 6 months and then held for 1 to 2 weeks at room temperature without developing excessive gas pressure, providing the conditions mentioned above are adhered to. When the calcium or magnesium hydroxide is present in the can the curing time may exceed a year. There appears to be a satisfactory margin of safety in this process to permit commercial usage providing the package is marked perishable to be held cold. It is obvious that gas formation by process cheese is not a problem in canning.

M22. Comparative Studies on Cheddar Cheese Prepared with Starter and with Certain Pure Cultures.[2]

D. D. DEANE AND T. G. ANDERSON, Dept. Bacter., Pennsylvania State College, State College, Pa.

All cheeses studied in this investigation were made by the usual commercial method from raw milk with a fat content of 3.6 to 3.7 per cent. Curing temperatures of 43° F. and 63° F. were used. The various cultures used as "starter" were *Str. citrovorus, Str. paracitrovorus, Str. lactis,* a commercial starter and an as yet unidentified acidoproteolytic micrococci isolated from a 4-year-old cheddar cheese.

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[1] Hiscox, Harrison and Wolf. Jour. Dairy Res., 12: 155. 1941.

[2] A phase of a cooperative study with C. D. Dahle and F. G. Warren of the Department of Dairy Husbandry.

By bacteriological study it was found that the highest counts were found when the first sample was taken when 1 day old. The number of microorganisms decreased steadily from this point on as the cheese aged. *Str. lactis* made from 90 to 99 per cent of the streptococci found which were predominate throughout the period the cheeses were examined. The temperature of curing had no significant influence upon the bacterial flora of these cheeses.

Str. citrovorus produced a cheese with a bitter flavor when used in conjunction with a commercial starter or a pure culture of *Str. lactis. Str. paracitrovorus* under these same conditions produced a slightly more desirable cheese from the standpoint of flavor. In conjunction with a commercial starter the flavor score remained at a comparatively high level, but this did not hold true when used with *Str. lactis.* There was no indication that the curing period could be significantly shortened by the use of any of the above pure cultures of streptococci as starters.

Cheese prepared with a commercial starter supplemented with the micrococcus isolated and used in this study received a flavor score after three weeks of from 0.5 to 2.0 points higher than the control cheese made with commercial starter. The body of the cheese prepared with the micrococcus is also superior after 3 weeks curing.

Studies on curing temperatures indicate that cheeses held at 63° F. for four weeks and then placed at 43° F., attain a higher flavor score sooner than cheeses held at 43° F. only. The flavor score of the higher temperature cheese was not, however, maintained as long nor was the maximum flavor score reached ever as high as that ultimately reached by the 43° F. cheese.

M23. General Action in Cheese of an Enzyme Preparation from Chicken Stomach. F. J. Babel, G. F. Stewart, and B. W. Hammer, Iowa Agr. Expt. Sta., Ames, Iowa.

An enzyme preparation that coagulated milk was prepared from the proventriculi of fowls for use in experimental cheese. Washed, minced glands were treated with an equal quantity of water and sufficient concentrated hydrochloric acid to obtain a pH of 1.5 to 2.0. After digestion for 18 to 24 hours at 40° C., an equal volume of ice water and 2 per cent talc were added. The mixture was allowed to settle at 0° C. and the supernatant liquid was removed. This was concentrated by evaporation at 25° C. or the enzyme solution was further purified by filtration and precipitation by saturation with sodium chloride. In special tests purified preparations clotted milk in a concentration of 1:250,000 and showed appreciable digestion of coagulated egg white in a concentration of 1:100,000. The enzyme solution was quite stable in liquid form.

When 35 ml. of the enzyme preparation per 100 pounds of milk was used in the manufacture of cheddar cheese, the milk formed a firm coagulum in approximately 30 minutes. During the cheddaring process the curd began to show evidence of proteolysis and the curd was somewhat soft. Progressive softening occurred throughout the ripening period. The cheddar cheese made with the enzyme preparation had a bitter flavor and the bitterness persisted throughout the ripening period.

Edam type cheese made with the enzyme preparation showed a rather soft coagulum as compared with rennet coagulation, but the curd acted normally. Cheese made by this method had a soft, smooth, waxy body. The cheese were rather bitter in the early stages of ripening, but the bitter flavor tended to disappear as the cheese were held.

THE THIRTY-SEVENTH ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

R. B. STOLTZ

Secretary-Treasurer

The American Dairy Science Association assembled in Fairchild Theater on the campus of Michigan State College in East Lansing, Michigan on Tuesday, June 23, 1942 at 9:00 A.M.

Past President Earl Weaver called the meeting to order and President Judkins introduced the officers of the Association.

Vice-President H. P. Davis then introduced the following Past Presidents: J. H. Frandsen, E. O. Anderson, A. A. Borland, O. E. Reed, J. B. Fitch, J. M. Sherman, E. L. Anthony, H. C. Jackson, R. B. Stoltz, H. A. Ruehe, H. W. Gregory, Earl Weaver, and E. S. Guthrie.

Secretary Stoltz introduced the following members who had served the Association as Directors: C. R. Gearhart, E. G. Hood, J. H. Nelson, and Harold Macy.

J. A. Hannah, President of Michigan State College, was then introduced and delivered the address of welcome.

President H. F. Judkins gave the following response:

PRESIDENT'S ADDRESS

"President Hannah, your words of welcome are very much appreciated although I can assure you that this group already feels very much at home on your delightful campus. Professor Weaver and his associates are proving themselves to be shining examples of Michigan hospitality. We know that when we leave you it will be with a desire to return. We shall endeavor to behave well while we are here. We wouldn't dare do otherwise as a glance at the map of your state shows that you have us right under your thumb.

"Even in these difficult times when everyone is busily engaged in an all-out war effort, progress demands that we keep abreaest of the times and also that we take an occasional furlough for recreation. This, the 37th Annual Meeting of the American Dairy Science Association, provides an opportunity for the leading minds in the dairy industry to take an inventory of the events of the past year and to renew that spirit of good-fellowship so characteristic of the meetings of this Association. I am glad so many have been able to take advantage of this opportunity in spite of summer teaching schedules and travel difficulties.

"I have said that this group represents the leading minds in the industry because a person must be a graduate of an agricultural college or hold a responsible position in the industry requiring a technical knowledge of dairying to be eligible for membership. Our active membership now numbers over 1200. About two-thirds of this number are engaged in milk production and processing and the balance are engaged in teaching, research and extension activities. In other words, one-third of our membership supplies the ammunition that keeps the remaining two-thirds forging ahead. Our program, consisting of scientific reports and practical symposia will, I am sure, provide something of interest and value to all.

Position of the Dairy Industry in the War Effort

"I want to say a word concerning the place of the dairy industry in the war effort. As you doubtless know, scientific research has long since established the fact that milk and milk products are absolutely essential to the growth and well-being of the human race. Our army and navy officials have placed milk and its products at the very top of the "must" items in the ration of the armed forces of our country. The vast population of this country who are now working harder than ever in an all-our war effort must take every precaution to preserve their health. Therefore, they need a liberal supply of dairy products more than ever.

"We who are assembled here should feel fortunate that we are engaged in an industry which is so essential to the war effort. Let's remind ourselves of this fact if we wonder, from time to time, whether we are doing all we can or what we should be doing to help win the war. Certainly any person who is trained or who is training others in the production, processing and handling of milk and its products and who is now employed in the dairy industry has every reason in the world to remain in that industry with a satisfied conscience that they are a very important cog in the war machinery.

Industry Adjustments Caused by the War

"The war has naturally caused many adjustments in the educational program of our colleges and universities as well as on the dairy farm and in the milk processing industry.

"In order that more students may complete their college course before entering the service of their country, many universities, including state universities and agricultural colleges, have speeded up their program so that students may attend the year round and complete their course in two and two-thirds to three years. Some who might normally be attending this meeting are now busy in the classroom or laboratory. If the war should continue through another year, the opportunity for work in the industry will be so great that college enrollment is likely to be further reduced unless young people are drafted by the government and required to attend college at least until of military age. This plan has already been suggested.

"The war is changing to some extent the type of dairy research projects. Thus, emphasis is placed on such work as raising calves with the minimum amount of milk, obtaining the maximum output per cow with the minimum input, industrial uses for milk by-products such as casein fibre, the possible use of various whey products in making lacquer coating as a substitute for tin, in making a rubber substitute, and in making a glycerine substitute, all of which projects hold promise. Emphasis is also placed on improved methods of manufacturing, packaging and storing such products as milk powder, evaporated milk and butter, all of which are greatly needed by the armed forces of the United Nations and will continue to be needed by the world population after the war is over. Government requirements for cheese, evaporated milk and powdered skimmilk have caused a marked increase in milk production and a shift in milk processing operations notably from butter making to cheese making.

"Lend-lease dairy supplies delivered to representatives of the United Nations since April 29, 1941 when the program started, to April 1, 1942 were as follows: Cheese, 163,953,774 pounds, Dry Skimmilk, 37,531,974 pounds; Evaporated Milk, 577,486,469 pounds. June milk production in this country is expected to reach 5,800,000,000 quarts, the largest monthly total ever recorded. The 1942 production may reach the all-time record of 56,000,000,000 quarts compared to a 1936–1940 yearly average of 49,000,000,000 quarts.

"Fresh milk and dairy products are being used in increasing quantities by the armed forces. Fresh milk is served daily to soldiers for the first time in history. Distribution of milk to the army is a man-sized job that is being successfully performed by the industry. Munitions plants are increasing the distribution of milk between meals as a means of decreasing fatigues and strengthening workers. The importance of milk in war time is further emphasized by the British experience where milk distribution has been carried on despite bombs and blitz.

"Prices received by United States dairy farmers are at the highest levels since 1930. Farm cash income from milk in 1941 topped all previous highs totaling $1,859,783,000. Present estimates indicate 1942 will total more than $2,000,000,000, an all-time high record.

"The milk and milk products industry is endeavoring to cooperate 100% with the various government agencies although it causes much burning of midnight oil to figure out ways and means of carrying on under the several government orders and recommendations. As of May 26 more than sixty orders, regulations or recommendations, all having some effect on the dairy industry, had been issued. Others, of course, may be expected as the situation changes. The effects on the dairy industry of some of the orders or recommendations are as follows: Effective as of June 1 it became necessary to take steps resulting in at least a 25% saving in mileage. This has meant radical changes, particularly in the distribution of milk and ice cream where many a horse and wagon is replacing a truck and where every-other-day delivery has become quite a common practice. New tires are, of course, out of the question, and the extent to which delivery services may be affected if the war is prolonged cannot be foreseen at this time. The ice cream manufacturer is now limited, on a two months' basis, to 70% of the cane or beet sugar used in the equivalent two months of 1941. There have also been some recent limitations on the amount of honey available for use in ice cream. To date, through changes in formula, the ice cream manufacturer has been able to get along successfully by using refined corn sugar and syrups. Processors of cocoa beans are permitted to grind, in any one month, only 70% of the quantity of beans ground in the similar month of 1941. As inventories are reduced, the ice cream industry will feel the effects of this order. There is a question as to whether dairy plants will, especially on the eastern seaboard, be able to get enough fuel oil to operate their boilers. This is causing some change from oil to coal and the building up to stockpiles of coal by these plants as well as by those now using coal. For some time aluminum has not been available for dairy equipment or bottle closures.

"A price ceiling—the maximum prices charged to the same class of customer during the month of March, 1942—has been placed on the retail sales of fluid milk and fluid cream and on wholesale and retail sales of ice cream. All standard grades of milk, flavored milks and cultured milks and various types of cream, as well as all flavors and grades of ice cream, sherbets and novelties, etc. come under the price ceiling. New or used plant equipment cannot be bought or sold without the specific approval of the Director of Industry Operations if it has a value of $300 or more. Plant equipment may be repaired and maintained in working condition without expansion or improvement through the use of preference ratings. Buildings may be repaired and maintained in working condition without expansion or improvement up to an expenditure of $5,000 in any twelve months' period. If the amount exceeds this, approval of the War Production Board must be had before beginning the project. Electric cabinets for the storage of ice cream, milk or other frozen foods may not be sold or installed except upon approval of the War Production Board. Fourteen and one-half-ounce cans for evaporated milk are available since evaporated milk is classified as a primary product. The 6-ounce cans, however, are restricted to a fraction of the 1940 use of these cans. Retinning of milk or ice cream cans is still permitted.

"I do not wish to leave the impression that the industry is complaining of its lot, but these examples will serve to illustrate that in our industry 'Business is not being carried on as usual.'

The College Graduate's Future in the Dairy Industry

"For the balance of my time I want to discuss the role of the college graduate in the dairy industry. My twelve years of teaching experience plus my experience in interviewing and working with college graduates in dairying for more than fifteen years has caused me to be greatly interested in this subject. While you who are teachers have a great opportunity and responsibility in training men for the dairy industry, the industry has an equally great responsibility in the proper selections of these men and in training them for responsible positions. During the past year while serving as your president I have attempted through correspondence and addresses to help improve the understanding on the part of both teacher and plant operator concerning the opportunities in the industry and the training and selection of men to take advantage of these opportunities. I talked on this subject at the meeting of the International Association of Ice Cream Manufacturers at Toronto last October. I have also appeared before students and faculty groups at Rutgers, University of Maryland, University of Minnesota, Iowa State College, University of Wisconsin, University of Illinois, Purdue University, Pennsylvania State College and Ohio State University. The list might have been longer had the times been more normal. I want to say that I very much appreciate the hospitality shown me on the occasion of these various visits. I enjoyed them thoroughly.

"The two L's, labor and legislation, have in the last ten years greatly increased the need of college trained men in the dairy industry. May I simply call to your attention some of the principal opportunities other than those of teaching, research and extension work that exist in our industry. Some of those in the production field are dairy farm managers, dairy farm owners, feed manufacturing and sales, breed association work, milk producers organization work and the publishing field. To take advantage of these opportunities one needs to have a full understanding of the feeding, breeding and veterinary care of dairy cattle. Among other things he needs supporting courses in the raising of crops, economics, and in milk processing.

"Some of the opportunities for key positions in the milk products field in addition to teaching, research and extension work are inspectors and sanitary engineers for the Federal government, State and City governments and commercial concerns, State Dairy Commissioners, the manufacture of dairy equipment, the sale of dairy equipment, work with the National Dairy Council, trade association work in the various branches of the dairy industry and dairy periodical publishing. In the commercial dairy business the following are some of the more important opportunities: research and laboratory technicians, plant superintendents or production managers and purchasing agents, office managers including the supervision of such departments as accounting, auditing, insurance, tax and treasury, legal advisers, company presidents and business owners. It will be observed that the above mentioned positions call for different types of minds and minds trained along a variety of different lines. The work, for example, of a plant superintendent is quite different from that of a man engaged in the sale of dairy equipment. In a like manner the work of an engineer is quite different from that of a sales manager and the work of a laboratorian is quite different from that of a lawyer or an accountant.

"It becomes increasingly evident that the college course in the junior and senior years should be quite flexible and that the head of the dairy department has a real opportunity to offer vocational guidance in order that each of his students may take that combination of courses which best fits his particular aptitude. Even then it appears probable that the industry may expect to select some of its men from schools of engineering, law or business administration. I am certain, however, that if men from such schools can have had some dairy work when they enter the industry they will find it very much to their advantage. It seems to me, therefore, that no time or opportunity should be lost in our state colleges and universities for the dairy department and various other departments mentioned above to cooperate to the fullest extent, helping to train the men needed in the industry. Some students majoring in economics or the school of business administration will do well to elect some undergraduate courses in dairying or do graduate work whereby their training in business is applied in the dairy field. There is less opportunity for the men majoring in the sciences and engineering to take undergraduate courses in dairying but some of these men will do well to pursue graduate work and at that time to take some courses in dairying and apply their training in this field. The industry can well afford to use more of these five, six and seven year men so trained.

"I understand dairy departments are requiring less vocational training in the laboratory for four-year men and I certainly believe that this is a step in the right direction. As a matter of fact, many four-year dairy graduates seem to be better at working with their hands than with their heads. I notice that even after some experience, they frequently do not have the knack of planning a day's production schedule, or the ability to plan an attack looking toward the solution of a plant operating problem or a product quality problem. Perhaps the reasons lie in the following statements quoted from a recent letter from one of our members who is engaged in teaching and research work. He states:

(1) 'Too many of our students are trained to accept a series of recipes for the solution of their problems rather than to exercise judgment in the application of sound, scientific principles to existing and new problems.'

(2) 'Too many of our students accept their college degrees as a trademark of a finished product rather than a token of an educational apprenticeship in a professional career, the success of which in a progressive industry is dependent upon eternal alertness.'

"Need I say that everything possible should be done to help develop the student's personality since this is likely to count for as much or more in his success than what he learns from the textbook. In this connection I should be remiss if I did not point out one weakness of many dairy graduates. It is that they are not careful enough about their personal dress and habits and that they lack the knack of being a good plant housekeeper. I know of no one thing that so much stands in the way of a man's success in dairy plant work. Teachers and dairy instructors will do well to bear down on this side of the student's training and if the student simply cannot be made to appreciate cleanliness, the suggestion had better be made that he major in vegetable gardening where he can really get right down in the dirt.

"Someone has said 'A college education makes a good man better and a fool a bigger fool.' This at once implies that industry has a job in selecting the wheat from the chaff when employing these men and by no means should we condemn the whole lot because we sometimes get a dud. There is certainly room for improvement in the selection and developing of college graduates in the average dairy company. As I see it, this is due to two things: First, the dairy industry consists of a very large number of relatively small operating units. There are no General Electrics, Westinghouses, A. T. & T.s or U. S. Steels in the dairy business; hence the need for college trained men per operating unit is less in our industry and what is more significant we do not have the personnel machinery for selecting and training the men that is used by the large companies.

"In the second place, when hiring a college graduate, many dairy executives do not, unbeknown to the graduate, have a responsible job in the company in mind which he expects said graduate eventually to fill if he develops properly. In other words, the hiring of these college men is more or less hit and miss, and frequently quantity rather than quality is employed.

"I cannot overemphasize the importance of interviewing men before they graduate and of trying to select the best for the jobs in question. Summer employment may well be used as a test of the ability of those selected. Having selected the man, the employer should really get acquainted with him and not proceed to forget him as soon as he is hired. Obviously the employer cannot make his plans for this man too obvious, neither can he afford to pamper him in any way. On the other hand, a little advice and guidance once in a while will often help to develop a good man that might otherwise get lost in the shuffle.

"I strongly recommend the use of an employee experience record which will be printed as a part of this address. By the use of this record a man is graded every six months or so during the early years of his employment and the record speaks for itself. If it is not good he will be dropped before he has wasted the best years of his life getting nowhere. If it is good, he will be advanced as fast as the opportunity permits. It is the only sensible plan for both the employer and the employee.

"In my visits to the several institutions seniors in dairying have asked me what their chance was likely to be in the dairy industry since they were going to have to enter the service of their country. My reply has been that other things being equal the maturing effect of their life in the armed forces would be sure to be an asset to them and that they would have this advan-

EMPLOYEE EXPERIENCE RECORD*

(Ice Cream Business)

Company - - - - Plant - Date -
Name of Employee - Age - Height Weight Married or Single .
Education and Experience before Employment

	Jan.	Feb.	Mar.	Apr. May June	July	Aug.	Sept.	Oct.	Nov.	Dec.
WORK CLASSIFICATION Date										
1. Procurement of raw materials				22. Accounting						
2. Receiving and handling raw materials				TYPE OF WORK DONE:						
3. Preparation of flavors and colors				1. Laborer						
4. Mixing				2. Foreman						
5. Laboratory work				3. Superintendent						
6. Freezing				4. Salesman						
7. Novelties				5. Plant Manager						
8. Fancy department				QUALIFICATIONS & QUALITY OF WORK DONE:						
9. Care of hardening room				1. Honesty						
10. Container washing				2. Employee relationships						
11. Cleaning equipment				3. Punctuality						
12. Power plant and refrigeration				4. Health						
13. Equipment maintenance				5. Energy						
14. Laundry operation				6. Thoroughness						
15. Shipping				7. Originality						
16. Delivery				8. Cost Mindedness						
17. Selling				9. Personal appearance						
18. Garage operation				RATE OF PAY:						
19. Cabinet department										
20. Stock room maintenance				REMARKS:						
21. Purchasing										

* Employees record should be examined by consulting his supervisor at least every 6 months for the first 2 or 3 years. Opposite date write period covered between such examinations such as 1/1/41 to 7/1/41. For each period place an (X) in the space opposite the work that occupied major part of time and a ( ) for part time work. At each period check employee's classification under "Type of Work Done." Mark G—good, F—fair, P—poor for each period opposite "Qualification and Quality of Work" items. Note opposite "Rate of Pay," rate of pay at starting date and note date when any change made and the new rate.

tage over the younger men graduating at about the time they get out of service. I am sure that the industry will not forget them when they can change the khaki or blue for the white uniform once more."

O. E. Reed, Chief of the Bureau of Dairy Industry and who was head of the Dairy Department at the host institution fifteen years ago when the Association held its meeting there in 1927, gave an address, "The Foster Mother."

There were 212 members present. The meeting adjourned at 11:20.

GENERAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

EAST LANSING, MICHIGAN, JUNE 25, 1942

President Judkins called the meeting to order at 3:30 P.M. in Fairchild Theater, there being 114 present. Secretary Weckel of the Manufacturing Section presented the following report. Upon motion duly seconded the report was accepted.

MANUFACTURING SECTION

Papers Presented: The papers scheduled on pages 13–15 of the Program were presented as listed with the following exceptions: Paper A (June 23 section) was presented by J. F. Devine of the War Production Board at the request of Clyde Beardslee. Papers M-7, M-13, and M-15 were omitted due to absence of the speakers. Paper A (O. A. Ghiggoile, June 24) was read by E. H. Parfitt. Under A (June 24) the Chairman invited Commander A. W. Fuchs to speak extemporaneously on the operation of the dairy section of the U. S. Public Health Service.

Business meeting, June 24, 1942: L. H. Burgwald presided.

Burgwald presented a request of the Board of Directors of the Association that the Manufacturing Section consider a change in the tenure of officers. They recommend that the term of office expire July 1 of each year. Motion was made by O. F. Garrett and seconded by E. F. Hansen that newly elected officers of the Manufacturing Section assume office beginning July 1 of each year. Motion carried.

Report of Committee on Reorganization of Committees: Burgwald presented a report of the Committee on Reorganization of Committees, presented by R. Whitaker. Acceptance of the report (Appended) was moved by M. E. Parker and seconded by A. W. Farrall, and unanimously adopted.

On Election of Officers: H. Macy suggested that the method of election of officers of the Manufacturing Section was not satisfactory in that the election was held at the termination of a day's meeting when attendance was not representative. He suggested further that it would be preferable to have the election of Section Officers by mail ballot at the time ballots for election of Association Officers are submitted to the entire membership. It was moved by H. Macy and seconded by M. E. Parker that a Committee of three be appointed to study this matter in collaboration with officers of other sections. Motion carried. Chairman Burgwald appointed a Committee on Election of Manufacturing Section Officers consisting of H. Macy, H. H. Sommer, and E. S. Guthrie to study the matter and to report at the next annual meeting of the Society.

Election of Manufacturing Section Officers: C. J. Babcock, Chairman, read the report of the Nominating Committee. The report was accepted. Paul F. Sharp, Cornell University, was elected vice-chairman and R. J. Ramsey, Cleveland, Ohio, was elected Secretary. Vice-Chairman R. Whitaker by rule, automatically becomes Chairman of the Manufacturing Section.

Report of Business Meeting, Manufacturing Section, Thursday, June 25, 1942: R. Whitaker proposed an amendment to the Report of the Committee on Reorganization of Committees of the Manufacturing Section as follows: Creation of two additional committees; a Committee on Evaporated Milk; a Committee on Dried Whole and Dried Skimmilk; and further, that all products not specifically mentioned for listed committees be included in products under direction of the Committee on By-Products. The motion was seconded by H. Macy and unanimously adopted.

Report of Committee on Chemical Methods of Analysis: L. C. Thomsen presented the report of the Committee on Chemical Analyses appended herewith. Thomsen recommended that in the reappointment of committee members that the Committee (Subcommittee) on Skimmilk, Buttermilk and Whey be continued as it now exists so that work now in progress may be brought to completion. It was moved by Thomsen and seconded by Nelson that the report of the Committee be adopted. This was unanimously adopted.

Report of the Committee on Judging of Dairy Products: E. L. Fouts presented the report of the Committee on Judging of Dairy Products, appended. Fouts moved acceptance of the report, which was seconded, and unanimously adopted.

Report of the Committee on Score Cards for Dairy Products: C. J. Babcock presented the report of the Committee on Score Cards for Dairy Products, appended. Acceptance of the report was moved, seconded, and unanimously adopted.

Report of the Committee on Oxidized Flavor: No report.

Report of Committee on Methods of Determining Color of Milk: No report.

Report of the Committee on Comparison of the Gerber and Babcock Tests: J. H. Erb presented the report of the Committee on Comparison of the Gerber and Babcock Tests, appended. Adoption was moved, seconded, and unanimously adopted.

Report of the Committee on Quality Program: P. H. Tracy presented reports of subcommittees on the Quality Program. The report of the subcommittee on Cream, appended, was presented by H. C. Olson. The subcommittee on Ice Cream submitted no report. The report of the subcommittee on Cheese, appended, was presented by W. V. Price. The subcommittee on Condensed Milk and Milk Powder submitted no report. The report of the subcommittee on Milk Quality was prepared by H. A. Bendixen (report appended) and discussed by P. H. Tracy.

Tracy moved, that the reports of the Committee on Quality Program be accepted. Motion was seconded and unanimously adopted.

Meeting adjourned June 26, 1942.

PRODUCTION SECTION

Secretary Dwight Espe of the Production Section then gave the following report. On motion duly seconded the report was adopted.

The Production Section held three joint meetings with the Extension Section and one with the Manufacturing Section. On Wednesday, June 24th, papers of special interest, but relating to different fields, were presented on two programs. These programs were presided over by H. A. Herman and K. E. Turk, Chairman and Vice-chairman respectively of the Production Section. The largest total attendance during the day was 130.

The Section held two business meetings, each of which was presided over by Mr. Herman.

Reports from the various standing committees were called for. Copies of these reports are attached. Salient points incorporated in these reports are presented herewith for approval.

1. *Breeds Relation Committee:* The Breeds Relation Committee met with representatives of the various dairy breed organizations. The committee voted: (1) to approve the adoption and use of the Uniform Dairy Score Card; (2) to approve the recommendations of the Purebred Dairy Cattle Association committee on Standardizing Testing Rules and Methods, *with the exceptions* noted on the attached copy. (3) It is further recommended that during the present emergency the minimum requirements with respect to the number of tests required for acceptance and publication of records as contained in the recommendations of the Testing Committee of the Purebred Dairy Cattle Association be approved. (4) That the recommendations of the Committee on Uniform Rules and Methods for Proving Sires and Brood Cows as per attached copy, be accepted.

The report of this committee was approved by the Production Section. The Section voted, with the approval of the Extension Section, to make the Breeds Relation Committee a joint committee from the Production and Extension Sections.

2. *Committee on Measuring Results of Pasture Investigations:* The com-

mittee asked that the American Dairy Science Association go on record as favoring early publication in the Journal of Dairy Science or a suitable journal the report entitled "Pasture Investigations Technique." The report referred to was submitted in December, 1940, by a joint committee from the American Dairy Science Association, the American Society of Animal Production and the American Society of Agronomy. The recommendation was approved by the Production Section.

3. *Committee on Awards for Students National Contest in Judging Dairy Cattle:* Since the recipients of the 1940 scholarships have been unable to accept these awards, due to active service in the war, the committee recommends that these scholarships be held open "for the duration."

4. *Committee on Methods for Proving Sires and Brood Cows:* Report of the committee was read and accepted. The Section voted to appoint a joint committee with the Extension Section to be called the "Committee on Methods for Proving Sires and Brood Cows."

5. *Committee on Silage Methods, Evaluation, etc.:* Report of the committee was read and approved.

6. *Resolutions Committee:* Report of the committee was read and approved.

The question was raised regarding the advisability of electing the officers of the Production Section during the business meeting or by mail. The Section was almost unanimous in voting to continue with the present plan of electing officers at the business meeting.

7. *Nominating Committee:* Report of the committee was read and accepted. G. W. Salisbury (Cornell) was elected secretary and Dwight Espe (Iowa), vice-chairman. K. L. Turk (Maryland), the present vice-chairman, automatically becomes chairman of the Production Section for the ensuing year.

EXTENSION SECTION

Secretary E. C. Scheidenhelm of the Extension Section then read the following report. Upon motion duly seconded it was adopted.

The annual meeting of the Extension Section opened on Tuesday afternoon, June 23 with a joint symposium session with the Production Section on the subject "Nutrition and Reproduction in Dairy Cattle."

The first business session was called to order June 24 at 9:00 A.M. by the Chairman, Glen Vergeront. Thirty members and sixteen guests were in attendance from 16 different states.

During the three day session the program was developed by the various committees within the section. In addition to the Extension dairymen represented on the committees a number of men had been drafted from the Production Section to supplement the program on various topics.

The report of the Testing Committee was presented by the Chairman, R. W. Dickson. Some of the more important recommendations of the committee in addition to the papers given were: (1) Whenever possible the service of the government employment agencies be used in locating possible men for DHIA work. (2) A cooperative grouping of associations be arranged in the states so that a continuous testing service can be maintained whenever possible. Report was accepted.

E. J. Perry presented the program of the Sire Committee. Two excellent papers were presented pertaining to artificial insemination work. A form is being developed by this group to be used by all artificial insemination work. Report of the committee was accepted.

G. E. Taylor presented the report of the Herd Health Committee. This report emphasized the need of a uniform program between states on health standards. Report accepted.

C. L. Blackman presented the feeding committee report. Report was accepted.

The Quality and Marketing Committee presented an excellent report which was accepted. A motion was passed to solicit the cooperation of the Quality Committee of the Manufacturing Section in holding a joint symposium of the Manufacturing and Extension groups at the next annual meeting of the Association on the subject of Quality Improvement of Dairy Products.

L. O. Gilmore presented the report of the recommendations of the Dairy Farm Management Committee which was to continue developing suitable standard forms for this project. Report accepted.

Reporting of the progress of the 4-H Dairy Club Committee was given by Joe Nageotte. Report of the Committee was accepted.

Progress of the Type Rating Committee was presented by E. C. Scheidenhelm. Report accepted.

The Exhibits Committee reported 11 states presented individual teaching methods and then a joint exhibit was presented by the Dairy Farm Management Committee. Report accepted.

During the business session the final report of the Resolutions Committee was accepted, and presented to General Resolutions Committee.

Two motions were passed to improve the committee work in the Production and Extension Sections. They were: (1) That there shall be a joint committee of the Production and Extension Sections known as the Dairy Cattle Breeding Committee rather than the present title of Sire Committee in both sections. The committee should consist of 6 men, 3 from each section. The chairman of each section to appoint the men representing his section. The chairman of the committee is to be appointed by the joint action of both sectional chairmen. '(2) That the Breed Relations Committee be a joint committee of three men from each section, they being appointed by the chairman of their section.

Mr. G. G. Gibson was unanimously elected Secretary of the Section. The officers for the following year will be J. F. Kendrick, Chairman; E. C. Scheidenhelm, Vice-Chairman; and G. G. Gibson, Secretary.

NECROLOGY COMMITTEE

Mr. Charles Blackman, Chairman of the Necrology Committee, reported the death of the following members during the past year: Lawrence H. Addington, William A. Kyle, R. C. Fisher, Merrill J. Mack, R. S. Fleming. Information regarding the activities of these deceased members was contained in the report of the committee. Upon motion duly seconded the report was accepted to be made a matter of record in the minutes.

REGISTRATION COMMITTEE

Mr. G. M. Trout, Chairman of the Committee on Registration, gave the following report:

Final tabulation showed the total registration at the 37th annual meeting of the Association to be 566 consisting of 394 men, 93 women, and 79 children. Classification of the men showed that 196 or 49.7 per cent were from colleges, experiment stations, and the United States Department of Agriculture, and that 198 or 50.3 per cent were from commercial laboratories, plants, state departments and inspectional staffs. Representatives were present from 38 states, the District of Columbia, and 2 provinces of Canada. The 12 leading states, including the District of Columbia, in representation were:

Michigan	184	Missouri	30	Washington, D. C.	13
Ohio	54	New York	29	Indiana	10
Illinois	51	Pennsylvania	20	Minnesota	9
Wisconsin	49	New Jersey	15	Kentucky	8

Inasmuch as the 37th annual meeting of the American Dairy Science Association was the 16th annual summer meeting of the Association and the second summer meeting held at Michigan State College, it seemed desirable to ascertain the number of meetings that the various members had attended. Consequently, when the letter seeking reservations was sent out a notice was sent also of the meeting places of the Association since 1906. Those anticipating attending the 1942 meeting were asked to check the meetings attended and return this sheet for compilation. Much interest was manifest in the meeting places. Approximately 140 replies from those expecting to attend the 1942 meeting were received.

The attendance at previous meetings of those reporting and who were present in 1942 is presented in Table 1. These data show the relatively high percentage of attendance at previous summer meetings of a cross section of 138 members attending the 1942 meeting and the influence of location of meetings on the attendance of those members. Only one member, E. S. Guthrie, who was present at the first meeting in 1906 was present at the

1942 meeting. Three members, J. H. Frandsen, C. Larsen, and O. E. Reed, attending the second meeting in 1907 were present at the 1942 meeting.

TABLE 1

The number and per cent of 138 members attending the 1942 ADSA meeting who attended previous meetings. Report based on 138 replies to questionnaire and not on total number in attendance at 1942 meeting.

Meeting	Location	Date	No.	Per cent
1st.	Urbana, Ill.	1906	1	0.7
2nd.	Chicago, Ill.	1907	3	2.2
3rd.	Ithaca, N. Y.	1908	4	2.8
4th.	Milwaukee, Wisc.	1909	5	3.6
5th.	Chicago, Ill.	1910	3	2.2
6th.	" "	1911	6	4.3
7th.	" "	1912	6	4.3
8th.	" "	1913	7	5.0
9th.	" "	1914	7	5.0
10th.	San Francisco, Calif.	1915	2	1.4
11th.	Springfield, Mass.	1916	9	6.5
12th.	Columbus, Ohio	1917	8	5.7
13th.	" "	1918	10	7.2
14th.	Chicago, Ill.	1919	19	13.7
15th.	" "	1920	20	14.4
16th.	St. Paul, Minn.	1921	25	18.1
17th.	" " "	1922	25	18.1
18th.	Syracuse, N. Y.	1923	29	21.0
19th.	Milwaukee, Wisc.	1924	24	17.4
20th.	Indianapolis, Ind.	1925	28	20.2
21st.	Detroit, Mich.	1926	34	24.6
22nd.	East Lansing, Mich. (1st summer)	1927	34	24.6
23rd.	Madison, Wis.	1928	36	26.1
24th.	Washington, D. C.	1929	40	29.0
25th.	Ames, Iowa	1930	55	39.8
26th.	Berkeley-Davis, Calif.	1931	9	6.5
27th.	Lexington, Ky.	1932	51	36.9
28th.	Urbana, Ill.	1933	57	41.3
29th.	Ithaca-Geneva, N. Y.	1934	76	55.0
30th.	St. Paul, Minn.	1935	68	49.2
31st.	State College, Pa.	1936	89	64.4
32nd.	Lincoln, Neb.	1937	77	55.8
33rd.	Columbus, Ohio	1938	100	72.4
34th.	Moscow, Idaho,-Pullman, Wash.	1939	45	32.6
35th.	West Lafayette, Ind.	1940	100	72.4
36th.	Burlington, Vt.	1941	97	70.3
37th.	East Lansing, Mich.	1942	138	

The members who have attended ten of *all* the meetings of the Association are: Fordyce Ely of Kentucky, B. E. Horrall of Indiana, Floyd Johnson of Iowa, H. B. Monier of Kentucky, F. B. Morrison of New York, T. S. Sutton of Ohio, and H. E. Herman of Missouri.

Those who have attended eleven of all the meetings are: E. O. Anderson of Connecticut, C. L. Blackman of Ohio, L. H. Burgwald of Ohio, J. C. Hening of New York, H. G. Lindquist of Mass., J. A. Nelson of Montana, Earl N. Shultz of N. H., Paul F. Sharp of New York, H. L. Templeton of Nebraska, L. C. Thomsen of Wisconsin, and G. M. Trout of Michigan.

Those who have attended twelve of the meetings are: R. E. Horwood of Michigan and G. E. Taylor of N. J.

Those who have attended thirteen of the meetings are: H. P. Davis of Nebraska, E. G. Hood of Canada, H. C. Jackson of Wisconsin, C. F. Monroe of Ohio, L. S. Palmer of Minnesota, and K. G. Weckel of Wisconsin.

Those who have attended fourteen of the meetings are: W. E. Krauss of Ohio, H. Macy of Minnesota, E. H. Parfitt of Illinois, W. V. Price of Wisconsin, C. W. Turner of Missouri and R. Whitaker of New York.

Those who have attended sixteen of the meetings are: R. B. Becker of Florida, S. J. Brownell of New York, A. C. Dahlberg of New York, H. O. Henderson of West Virginia, C. F. Huffman of Michigan, and S. M. Salisbury of Ohio.

Those who have attended seventeen of the meetings are: T. W. Gullickson of Minnesota, E. M. Harmon of Illinois, I. W. Rupel of Wisconsin.

Those who have attended eighteen of the meetings are: Earl Weaver of Michigan, and C. A. Hutton of Tennessee.

Those who have attended nineteen of the meetings are: P. S. Lucas of Michigan, A. B. Nystrom of Washington, D. C., and W. E. Petersen of Minnesota.

Those who have attended twenty of the meetings are: A. C. Baltzer of Michigan, A. J. Cramer of Wisconsin, H. W. Gregory of Indiana, E. S. Guthrie of New York, C. Larsen of South Dakota, and W. H. E. Reid of Missouri.

H. W. Cave of Oklahoma attended 21 of the meetings.

A. C. Ragsdale of Missouri attended 24 of the meetings.

Those who attended twenty-five of the meetings are: A. A. Borland of Pennsylvania, J. B. Fitch of Minnesota, and R. B. Stoltz of Ohio.

O. E. Reed of Washington, D. C., attended 30 of the meetings.

J. H. Frandsen of Massachusetts attended 31 of the meetings.

Of the 59 listed as having attended 10 or more of the 37 meetings, J. H. Frandsen heads the list with 31 meetings and O. E. Reed, a close second with 30 meetings. J. H. Frandsen attended 23 of the first 25 meetings while C. Larsen attended 17 of the first 19 meetings.

The names of those attending 10 or more of the 16 summer meetings are given below. Those attending 10 of these meetings are: Fordyce Ely of Kentucky, J. C. Hening of New York, H. A. Herman of Missouri, B. E. Horrall of Indiana, R. E. Horwood of Michigan, C. F. Huffman of Michigan, Floyd Johnston of Iowa, H. B. Monier of Kentucky, C. F. Monroe of Ohio, H. B. Morrison of New York, T. S. Sutton of Ohio, and G. E. Taylor of New Jersey.

Those who have attended eleven of the summer meetings are: C. L. Blackman of Ohio, L. H. Burgwald of Ohio, H. C. Jackson of Wisconsin, H. G. Lindquist of Mass., P. S. Lucas of Michigan, H. Macy of Minnesota,

J. A. Nelson of Montana, L. S. Palmer of Minnesota, E. H. Parfitt of Illinois, Paul F. Sharp of New York, Earl N. Schultz of New Jersey, Hugh L. Templeton of Nebraska, L. C. Thomsen of Wisconsin, G. M. Trout of Michigan, and Earl Weaver of Michigan.

Those who have attended twelve of the summer meetings are: A. C. Baltzer of Michigan, S. J. Brownell of New York, H. W. Cave of Oklahoma, J. B. Fitch of Minnesota, J. H. Frandsen of Massachusetts, H. O. Henderson of West Virginia, A. B. Nystrom of Washington, D. C., W. H. E. Reid of Missouri, S. M. Salisbury of Ohio, and C. W. Turner of Missouri.

Those who have attended thirteen of the summer meetings are: R. B. Becker of Florida, A. J. Cramer of Wisconsin, A. C. Dahlberg of New York, E. S. Guthrie of New York, E. G. Hood of Canada, W. E. Krauss of Ohio, W. E. Petersen of Minnesota, W. V. Price of Wisconsin, A. C. Ragsdale of Missouri, O. E. Reed of Washington, D. C., I. W. Rupel of Wisconsin, and K. G. Weckel of Wisconsin.

Those who have attended fourteen of the summer meetings are: A. A. Borland of Pennsylvania, H. W. Gregory of Indiana, T. W. Gullickson of Minnesota, and R. Whitaker of New York.

R. B. Stoltz of Ohio has attended 15 of the summer meetings.

R. B. Stoltz heads the list of attendance at summer meetings, having attended 15 of the 16. Four others, A. A. Borland, H. W. Gregory, T. W. Gullickson and R. Whitaker have attended 14 of the 16 summer meetings. Honors go to R. B. Stoltz for having attended all of the last fourteen meetings. T. W. Gullickson follows closely with 13, while A. A. Borland, H. W. Gregory, W. E. Petersen, I. W. Rupel, K. G. Weckel and R. Whitaker each have attended 11 years without missing a meeting. A. A. Borland has the enviable record of having attended 25 of the past 27 meetings, missing only two from 1916 to 1942, inclusive.

This report necessarily centers about the 1942 registration. While a record of the number of meetings attended by all the members of the Association would be highly desirable it was next to impossible to make such a compilation. Doubtless the names of many members of long standing not present at the 1942 meeting should have been included in the above lists. Possibly the names of some members present in 1942 have been overlooked. To them apologies are offered. So much interest has been manifest in the meeting places of the Association and the records of attendance that it seemed this compilation was worthy of special effort.

EDITOR'S REPORT

The Editor begs to submit the following brief report to the Board of Directors of the Association:

1. *Summary of Journal Contents.*

A summary of the Journal contents over the past four years is presented in the accompanying table.

SUMMARY OF JOURNAL CONTENTS

	1938–39	1939–40	1940–41	1941–42
Number of original articles	88	94	97	101
Pages of original articles	760	826	892	938
Number of reviews	2	3	5	3
Pages of reviews	26	119	144	80
Miscellaneous	140	98	157	106
Students National Contest, Proceedings Annual Meeting, Announcements, Circulation, Index, Committee Reports.				
Pages of Abstracts	232	206	304	418
Total number of pages printed	1158	1245	1497	1542
Classification of Articles				
Manufacturing articles	52	53	56	59
Pages occupied by Manufacturing	482	450	514	548
Production articles	28	31	35	36
Pages occupied by Production	212	296	316	336
Manufacturing-Production	8	10	6	6
Pages occupied	66	80	62	54
Classification Reviews				
Manufacturing reviews			5	1
Pages occupied by Manufacturing Reviews			144	36
Production reviews				2
Pages occupied by Production Reviews				44

The figures presented in this table represent 11 issues of the Journal for each of the first 3 columns, the June issues not being included. Figures for the present year include 12 issues inasmuch as the present June issue is given over to the publication of original papers. The total size of the Journal has not increased the past year; in fact, it is some 40 pages less than 1940–41.

2. *Abstracts:* You will note a substantial increase in the number of pages of abstracts. During the past year a set of directions for abstractors and associate abstract editors was prepared. These directions were well received and should materially assist in promoting further improvement in our abstract service.

The advance abstract service was started following the sanction of the Board of Directors at the annual meeting of 1940. The principal reason for initiating this program was as follows. At that time we were considerably behind in the publication of manuscripts and it was usually six months or longer from the time a manuscript was received until it appeared in print. Out of courtesy to our contributors the plan was proposed to call attention to their work which would later be published in detail.

We have since managed to catch up on publication until at the present if a reviewer delays in returning an article which has been mailed to him for review the abstract may not appear until the same issue in which the manuscript appears. Therefore the major reason for undertaking this project is no longer valid. This project might be dropped without any serious curtailment of service to our readers or contributors. I so recommend.

3. *The Twenty-Year Index:* The Twenty-Year Index has appeared. Those who have examined this work are impressed with the magnitude of the undertaking and the masterful way in which the job was done. We wish to publicly express our gratitude and appreciation to Dr. Harold Macy to whom all credit for this lasting contribution is due.

To all of those who have given so generously of their time and energies in our assistance, we wish to express our gratitude. The continued success of our Journal is dependent upon this fine spirit of helpful cooperation.

Upon motion duly seconded the report was accepted.

JOURNAL MANAGEMENT COMMITTEE REPORT

A. A. Borland of the Journal Management Committee then read the following report:

Your Committee on Journal Management beg to respectfully submit the following report covering operations during the year 1941–1942.

Directions for Associate Abstract Editors and Abstractors: The Editor, in collaboration with the associate abstract editors, prepared a set of directions intended to serve as a guide to the abstractors. These directions have for their purpose to set forth a general plan of the work of abstracting, to state the requirements as to content, style, and form, and to improve the quality and uniformity of abstracts. The directions are replete with information that will prove helpful to the abstract editors and the abstractors in their efforts to comply with the editorial policies and standards of the Journal. We congratulate the editor on this worthwhile achievement.

Use of Journal Abstracts in Other Publications: It has come to the attention of our committee that at least in one case an abstractor has been following the practice of supplying the "Journal of Biological Abstracts" with copies of his abstracts made and intended for the Journal of Dairy Science. This was done without the knowledge of the editor or the Board of Directors. We believe such practice to be contrary to the best interests of our Journal and should be discontinued. We, therefore, recommend that your Board establish a rule embodying the sense of the following suggestions:

1. That in as much as abstracts, being paid for by our Association are the property of this Association and not of the paid abstractors, abstractors may not dispose of copies of their abstracts to other publications, and
2. That all requests from other publications upon our abstractors for copies of their abstracts shall be referred to the Editor.

Policy on Distribution of Reprints: There appears to be a tendency on the part of some of the committee chairmen to take charge of the distribution of reprints of their committee reports. This practice tends to complicate our system of handling reprints, to cause inevitable confusion in our reprint service. It appears desirable, therefore that a definite policy be established on the distribution of reprints of committee reports and of similar special material published in the Journal, preferably in line with the following suggestions:

1. That all orders for reprints of committee reports be referred to the. Secretary-Treasurer, and that the distribution of such reprints be handled in a similar way as is now being done with reprints on "Methods of Analysis."
2. That the Secretary-Treasurer invoice the recipients to the extent of fully covering the cost of the reprints.

Price of Journal to Countries Other than the United States and Canada: It has always been the practice of the Journal to charge fifty cents additional for subscriptions from all countries other than the United States, its possessions and Canada. This appears to be an unnecessary hardship, especially to subscribers in North and South America. Upon suggestion by the Secretary-Treasurer, your committee authorized elimination of this extra charge, subject to approval of your Board, making the subscription price of the Journal to all countries in North and South America the same as for the United States and Canada, *i.e.* $6.00 instead of $6.50. We respectfully request the approval of this action by the Board.

Recommendations by the General Board of Dairy Research: We appreciate the suggestions relative to abstract service, review articles, etc., contained in the 1942 Report of the Committee on Abstracts and Reviews of the General Board of Dairy Research, and it is our desire and our effort to cooperate with the General Board of Dairy Research to the best of our ability. In the present abnormal times, however, with a decline of our revenues from membership dues, subscriptions, and advertisements, and with an increase in the cost of issuing the Journal, we have reason to doubt the wisdom of making changes in the Journal set-up that would further increase its operating cost, unless additional revenues can be made available in amounts sufficient to cover the added cost.

Cancellation of Macfarland and Heaton Contract: On account of complete absence of any action on the part of the advertising firm of Macfarland and Heaton in soliciting advertising for the Journal as per their contract that was approved by your Board at last year's Annual Meeting, we recommend cancellation of our contract with the above agency, to take effect at once.

Financial: The financial performance of the Journal for the year 1941 has been satisfactory. Although the income from members and subscribers has been decreasing each year since 1939, and the cost of printing the Journal has gradually increased each year since 1935, the Journal made a net profit as shown by the annual report of the certified Public Accountant, a copy of which reached every member of the Board of Directors and the Journal Management Committee.

Elimination of Advanced Abstract Service: At the Annual Meeting of 1940, it was agreed that abstracts of papers which were to appear in the Journal should be abstracted for this reason: That it was usually six months or longer from the time an article was received until it appeared in print. By printing the abstract in advance, the readers would know what was to be published. Since we are now pretty well caught up on publications, the advance abstract frequently appears in the same Journal as the original article and the need of printing the abstract separately is not as important as it was when it was longer between receiving the article and the time of its publication. We, therefore, recommend that the printing of abstracts in advance of their publication in the Journals be discontinued in the interest of economy.

It was moved, and seconded, that the Journal Management Committee report be accepted.

SECRETARY-TREASURER'S REPORT

The Secretary-Treasurer gave the following report:

Membership and Circulation: The circulation of the Journal for 1941 excelled all previous years. It reached 2,417. In 1939 it was 2,400 and in 1940 it was 2,374. Last year our 2,417 circulation was made up of 1,292 members, 669 subscribers, 65 associate subscribers and 391 student affiliates.

In 1941 we took in 93 new members, 59 of which paid their $5.00 affiliation fee and 34 were student affiliates and became members without paying a fee. This year up to June 17 we had taken in 69 new members, 34 of which paid the $5.00 fee, and 35 who were former student affiliates.

Assuming that we will have 30 additional new members the balance of this year, we may count on an increase of 100 members each year. How does this compare to our losses? In 1940, 99 of our members were dropped from the rolls because of their being delinquent, 19 resigned their membership, and 5 died making a total loss of 123. In 1942 we have had 5 deaths, 49 resignations, and 130 members are now delinquent. The possibilities are that 30 of these members will still pay their dues, which will make a total loss of 154 members. This is just about 25 greater loss than it was in 1941, and our loss in members this year is estimated at about 50 more than new members we have received, making a net loss of about 50 members.

You may be interested as to which members become delinquent, whether they are new or old. Of the 130 members delinqunt 19 had only been members one year; 21 had been members for two years; 35 had been members for three years; 24 had been members for four years; 10 had been members for five years; and 21 had been members for five years or more.

This brings us to the problem of why men who have been trained in the science of dairying, make their living in this field but who are not sufficiently interested to sacrifice $5.00 per year so that they may continue to receive the Journal of Dairy Science. It is my opinion that during the student's four years in college he has not had impressed upon him the necessity of his continued need of reading to keep up to date.

The Secretary then read a letter from Professor Goss of Iowa regarding the need of assigning articles to be read in the Journal by the students. He commented that we are not making as much use of the Journal and its facilities among our students as the worth of our publication warrants. Professor Goss told in his letter about Weckel of Wisconsin writing to parents of students to have them give a student affiliation as a Christmas letter to their sons.

The Secretary read the number of subscribers from four of the twenty-four foreign countries to demonstrate that those countries who are at war have not decreased their interest in dairy science:

	1938	1939	1940	1941	1942
England, Ireland, and Scotland	55	50	52	46	44
Australia and New Zealand	49	50	50	54	53
Japan	48	45	40	38	.
U. S. S. R.	34	47	33	36	4

Illinois leads the states in memberships having had 127 members last year. New York leads with suscriptions with 47 last year and 49 this year. Iowa stands first in number of student affiliates both this year and last. They had 56 last year and 48 thus far this year.

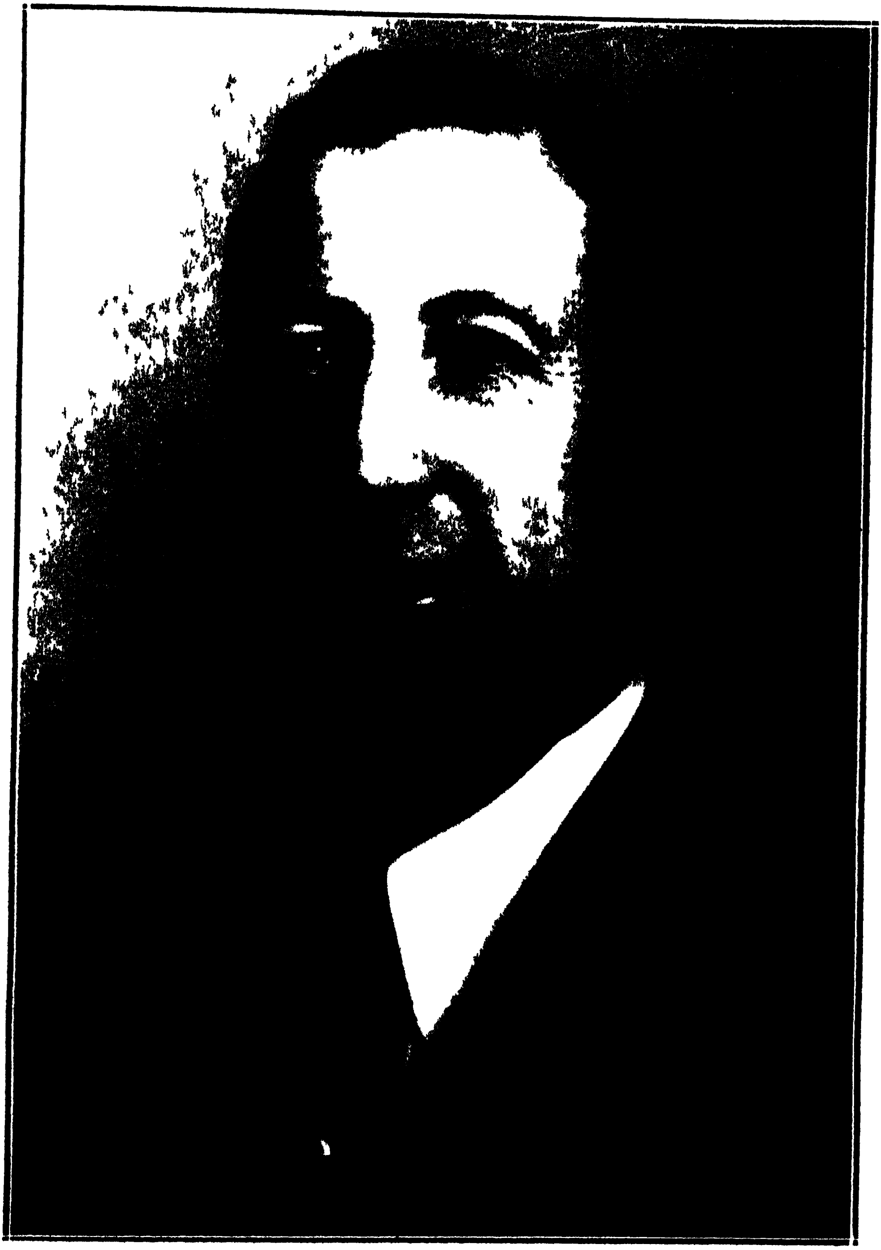
Index: 2,000 copies of the 20-Year Index were printed; 1,000 in paper covers and 1,000 in cloth covers. On June 17, 1942, we had delivered 706 paper covered copies, and 363 cloth covered copies.

These indexes cost approximately $4,000. We have received a little more than $1,500 so that the Association will probably be out about $2,000 and the Board of Directors made the price in this way because they believed this was a contribution that they could make to the membership.

All paper bound 20-Year Index are now $2.00, and cloth bound are $2.35; to non-members they will sell for $5.00 and $5.50.

Back Copies: Since the 20-Year Index is now in your hands it would be a good time to complete your back volumes. We suggest that you check your libraries and that they have the complete volumes from one to twenty-four inclusive.

Advertising: Our advertising has been most satisfactory. In 1938 we sold 93¾ pages; in 1939, 124 pages; in 1940, 126½ pages, and last year, 1941, 140¾ pages. The first half of 1942, we have sold 67 pages, which is less than 1941, but a little more than 1940. We are most grateful for those who use our pages as an advertising media. Last year our advertising income



PROFESSOR H P DAVIS, President Elect

amounted to more than $5,000.00 which is equivalent to the dues of 1,000 members. We will appreciate any courtesies shown the advertisers.

Financial: A report of the Certified Public Accountant was sent to each Director the latter part of February. This shows that our income for 1941 was $18,246.59, and our operating expenses were $17,566.03. Our net worth is $20,100.11. We have an investment in Government Bonds of $17,790.00 in addition to the $1,500 worth authorized by the Directors at their Board Meeting yesterday. This does not include income and expenditures of printing the 20-Year Index.

Upon motion duly seconded the Secretary's report was accepted.

The Secretary then read the minutes of the Board of Directors meetings. They will be found following the minutes of this meeting.

A motion was made and seconded that the minutes of the Board of Directors be accepted, and that all action taken by the Board of Directors during the past year be approved and endorsed by the Association. Motion carried.

S. M. Salisbury of the Auditing Committee submitted the following report:

May 16, 1942

To the Members of the American Dairy Science Association

Gentlemen:

Mr. Walter Burnham of Columbus, Ohio, Certified Public Accountant, has made an audit and report of the financial condition of the Association.

The Auditing Committee has conferred with Mr. Burnham and is satisfied that he has made a careful examination of all assets and liabilities of the Association and that all accounts are accurate. The committee is satisfied that the balance sheet and related summary of profit and loss represents the financial condition of the American Dairy Science Association.

Upon motion duly seconded the report was accepted.

Mr. C. G. Bradt of Cornell presented a report of the committee officially appointed by the American Society of Animal Production, the American Veterinary Medical Association, the Poultry Science Association, the U. S. Livestock Sanitary Association, and the American Dairy Science Association to discuss plans for cooperative work on animal disease and productive efficiency. Upon motion duly seconded the report was accepted.

Mr. Paul Sharp of Cornell who had been appointed by President Judkins to represent the American Dairy Science Association on the General Board for Dairy Research reported of their activities.

O. E. Reed, who had been appointed to represent the American Dairy Science Association on the National Research Council gave a report of the work of the Council.

NOMINATING COMMITTEE

Mr. H. C. Jackson of the Nominating Committee gave the following report. Upon motion duly seconded the report was accepted.

For Vice-President: A. C. Dahlberg and G. M. Trout
For Directors: R. B. Becker and Dwight Seath
W. B. Nevens and W. E. Petersen

RESOLUTIONS COMMITTEE

Mr. W. H. E. Reid, Chairman of the Resolutions Committee, submitted the following report:

The American Dairy Science Association assembled in its thirty-seventh annual meeting at Michigan State College, wishes to express for the members, their families and guests, its appreciation for the hospitality, delightful entertainment and splendid facilities provided by the officials and faculty of that college.

Therefore, be it Resolved: That the members of the Association publicly express their sincere appreciation to Dean E. L. Anthony; to Professor Earl Weaver and his departmental staff; to the Kalamazoo Vegetable Parchment Company; to the Michigan Dairy Industry Committee; to the Michigan Dairy Herd Improvement Associations; to the National Dairy Products Corporation; to the Borden Dairy Company and to the several departments of Michigan State College and other agencies cooperating in providing the entertainment and many fine courtesies.

Whereas: The general health and physical well-being of the men in our armed forces and those of our Allies and our people at home constitute the first essential in our national defense, and,

Whereas: We recognize the necessity to safeguard the health and well-being of the American people, that they be encouraged to use more milk, butter, cheese and ice cream and other dairy products consistent with the balance of our Lend Lease aid to our Allies.

Therefore, be it Resolved: That this Association urge the Honorable Claude Wickard to issue a statement encouraging the people of the United States to consume larger volumes of milk and milk products and thereby help to adjust existing conditions which now tend to jeopardize the entire dairy industry.

Whereas: The United Dairy Committee under the leadership of its capable chairman, Mr. Ralph Ammon, Commissioner, State Department of Agriculture of Wisconsin, has made definite progress in its endeavor to protect the dairy industry of this country,

Therefore, be it Resolved: That this Association commend and recognize the efforts of Commissioner Ammon and the members of the United Dairy Committee.

Whereas: Our Federal Government has asked for an increase in the total volume of milk as a part of the war program,

Therefore, be it Resolved: That we commend the notable efforts that our Dairy Extension Specialists have put forth by aiding the dairy farmers in approaching the national goals.

WHEREAS: The Bureau of Dairying and the Bureau of Home Economics of the U. S. Department of Agriculture, the Federal Food and Drug Administration; the Quartermaster Department of our armed forces; the U. S. Public Health Service; the Departments of Agriculture and the State Departments of Health of our respective states have made worthy contributions to the diet of our men in service, with special reference to milk and milk products,

Therefore, be it RESOLVED: That this Association commend these different federal and state agencies for their respective contributions.

WHEREAS: O. A. Ghigoile, Chief, Bureau of Dairy Service, California State Department of Agriculture; L. G. Kuenning, Chief, Dairy Division, Wisconsin Department of Agriculture; V. L. Fuqua, Dairy Commissioner, Tennessee Department of Agriculture; and Commander Fuchs, Director, Milk Division, U. S. Public Health Service, made able contributions to the program of this annual meeting,

Therefore, be it RESOLVED: That this Association express its sincere appreciation to each of these men.

WHEREAS: The work of a dairy herd improvement association is fundamental in furnishing a background of information for sound and efficient action in 4–H dairy club work, sire proving, dairy cattle breeding, selection and improvement,

Therefore, be it RESOLVED: That this Association hereby commend the work of the Bureau of Dairy Industry U. S. D. A., and the office of Dr. J. F. Kendrick, and that of the several states in this field and bespeak the continuance and the financial support for this work.

WHEREAS: The Purebred Dairy Cattle Association has developed (1) a Standardized Dairy Cow Score Card, (2) Standardized Production Testing Rules, (3) Uniform Rules and Method for Proving Sires and Brood Cows, and (4) Uniform Blanks for the Identification and Reporting of Registered Cows Artificially Inseminated in Cooperative Breeding Association,

Therefore, be it RESOLVED: That the American Dairy Science Association highly commend the Purebred Dairy Cattle Association for this progressive action.

WHEREAS: The demonstrations given at Michigan State College relating to experiments in progress created great interest in these investigations,

Therefore, be it RESOLVED: That other host institutions be encouraged to arrange for similar demonstrations when the nature of the experiments make that possible.

WHEREAS: This Association recognized the importance of awards in giving incentive to students throughout the country for continued activities and study in the field of dairy cattle breeding and production,

Therefore, be it RESOLVED: That donors of prizes for the winners in the National Collegiate Students Dairy Cattle Judging Contest be thanked individually in the name of the Production Section of this Association by the chairman of the Committee on Awards.

WHEREAS: This Association recognizes the importance of awards in giving incentive to students throughout the country for continued activity in study in the field of dairy manufactures, Therefore, be it RESOLVED: That donors of prizes for the winners in the National Collegiate Students Dairy Products Judging Contest be thanked individually in the name of the Dairy Manufacturing Section of this Association by the chairman of the Committee on Awards.

WHEREAS: The Borden Company is continuing its awards for recommendation of continued research in dairying,

Therefore, be it RESOLVED: That the American Dairy Science Association express its appreciation to the Borden Company for its continued interest in dairying.

WHEREAS: Dr. Harold Macy has made such an excellent contribution to the American Dairy Science Association by preparing the excellent 20-Year Index of the Journal of Dairy Science,

Therefore, be it RESOLVED: That this Association shall express its utmost appreciation to Dr. Macy.

P. F. Sharp moved and G. M. Trout seconded that the report be accepted.

Meeting was then adjourned.

MEETING OF BOARD OF DIRECTORS, AMERICAN DAIRY SCIENCE ASSOCIATION

R. B. STOLTZ, SECRETARY-TREASURER

East Lansing, Michigan, 8:00 P. M., June 22, 1942

A meeting of the Board of Directors of the American Dairy Science Association was held in the library of Abbot Hall Monday, June 22, 1942, at 8:00 P.M.

Present: President H. F. Judkins; Vice-President H. P. Davis; Secretary-Treasurer, R. B. Stoltz; Directors, Fordyce Ely, A. C. Dahlberg, J. C. Knott, G. M. Trout.

The three Sectional Chairmen, J. F. Kendrick, L. H. Burgwald, and H. A. Herman, were invited to meet with the Directors in order to discuss the rules and regulations of the sections.

The Manufacturing and Extension Sections were requested to change the time of incoming officers taking their office from October 1st to July 1st following their election.

The President was authorized to appoint a committee to submit amendments to our Constitution and by-laws. The following were appointed to report at our next annual meeting: A. C. Dahlberg, Chairman; G. M. Trout, and R. B. Stoltz.

Editor T. S. Sutton then presented the report which is printed in the minutes of the General Meeting, followed by the Journal Management Committee report which is also reported in the preceding minutes.

President Judkins then announced that O. F. Hunziker had submitted his resignation from the Journal Management Committee and the Secretary was authorized to notify O. F. Hunziker of the Association's deep appreciation for the many valuable contributions rendered during the many years of his service on the Journal Management Committee.

Davis moved and Dahlberg seconded that O. F. Hunziker be made a life member of the American Dairy Science Association as a token of our appreciation of his work on the Journal Management Committee.

President Judkins appointed A. C. Dahlberg to serve as chairman of the Journal Management Committee.

Ely moved, Davis seconded that the Pennsylvania State Student Branch be given a Certificate of Membership.

The Secretary-Treasurer then gave his report which is printed in the preceding minutes of the General Meeting.

The budget which was mailed previously to all Directors for the year 1943 was approved.

Since each member is given an opportunity to make nominations for the Borden Award at the time of election of officers there seems to be no need of nominating committees for this purpose, and it was therefore moved and seconded that the nominating committee for the Borden Awards be abolished.

The second meeting of the Board of Directors was held in the library of Abbot Hall Wednesday, June 24, 1942, at 11:15 A.M.

Present: President H. F. Judkins; Vice-President H. P. Davis; Secretary-Treasurer, R. B. Stoltz; Directors, H. W. Cave, Fordyce Ely, A. C. Dahlberg, J. C. Knott, and G. M. Trout.

The minutes of the last Board meeting were read and approved.

A letter was read from Director Chas. N. Shepardson stating that it would be impossible for him to attend the Annual Meeting and that it was with sincere regret that they would be unable to entertain our Association in 1943. This condition has been brought about by the accelerated program and the training and housing of fourteen hundred sailors in a naval radio school. He further requested that their invitation be kept on file and that the annual meeting be held in Texas as soon as possible after the termination of the present conflict.

An invitation was read from the University of Missouri to hold our annual meeting in Columbia, Missouri, in 1943.

Cave moved and Davis seconded that the invitation from the University of Missouri for 1943 be accepted.

It was moved by Trout, seconded by Ely that the Secretary-Treasurer be authorized to invest $1,500.00 in (2,000 worth face value) War Bonds Series F.

The board then adjourned.

AMERICAN DAIRY SCIENCE ASSOCIATION PRESENTED BORDEN AWARDS TO H. H. SOMMER AND W. E. PETERSEN

Michigan State College
Eost Lansing, Michigan, June 25, 1942

Mr. E. L. Anthony acted as toastmaster at the Annual Association Banquet, and presented Mr. W. A. Wentworth of the Borden Company who introduced six of the former recipients of the Borden Award: C. F. Huffman, W. E. Krauss, K. G. Weckel, C. W. Turner, Paul F. Sharp, and L. S. Palmer, who received the Award for the American Chemical Society.

Mr. H. W. Gregory, Acting Chairman of the Committee for the Borden Award in Dairy Manufacturers made the following statement:

"The Borden Awards to the American Dairy Science Association are made possible by the generous support which one of our largest corporations engaged in the Dairy Industry has seen fit to offer each year to two individuals for outstanding research pertaining to dairy production and dairy manufacturing. The Borden Company has insisted that in the administration of these awards, that this Association have complete jurisdiction over the rules covering the method of selection of each recipient, the naming of the recipient, and methods of presentation of the awards.

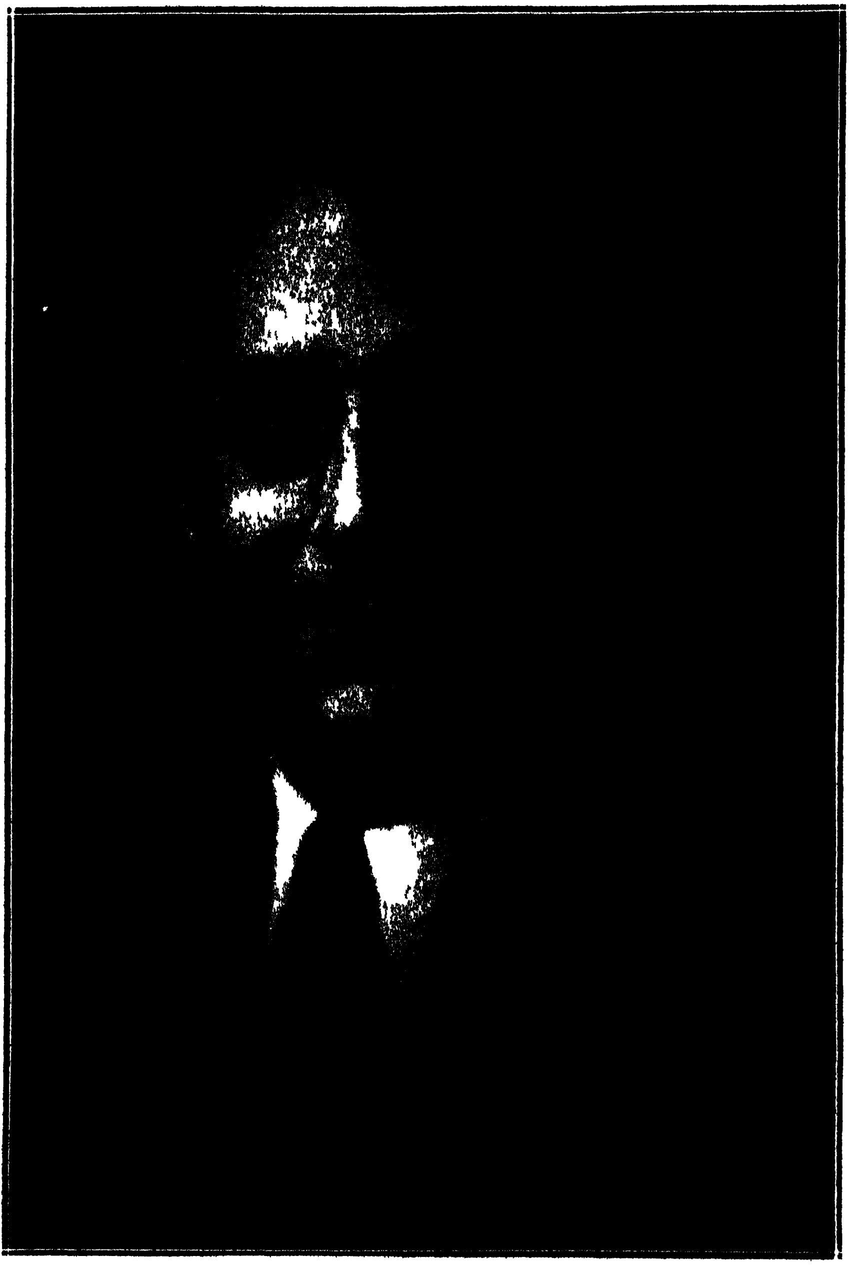
"Each of the awards consists of a gold medal and one thousand dollars ($1,000.00). The Borden Company has offered these awards since 1936 and the first Borden Award was made in 1937.

"Acting for the Chairman, Dr. N. W. Hepburn of the Dairy Manufacturers Borden Award Committee, it is my privilege and duty to name the recipient of the Dairy Manufacturing Research Award.

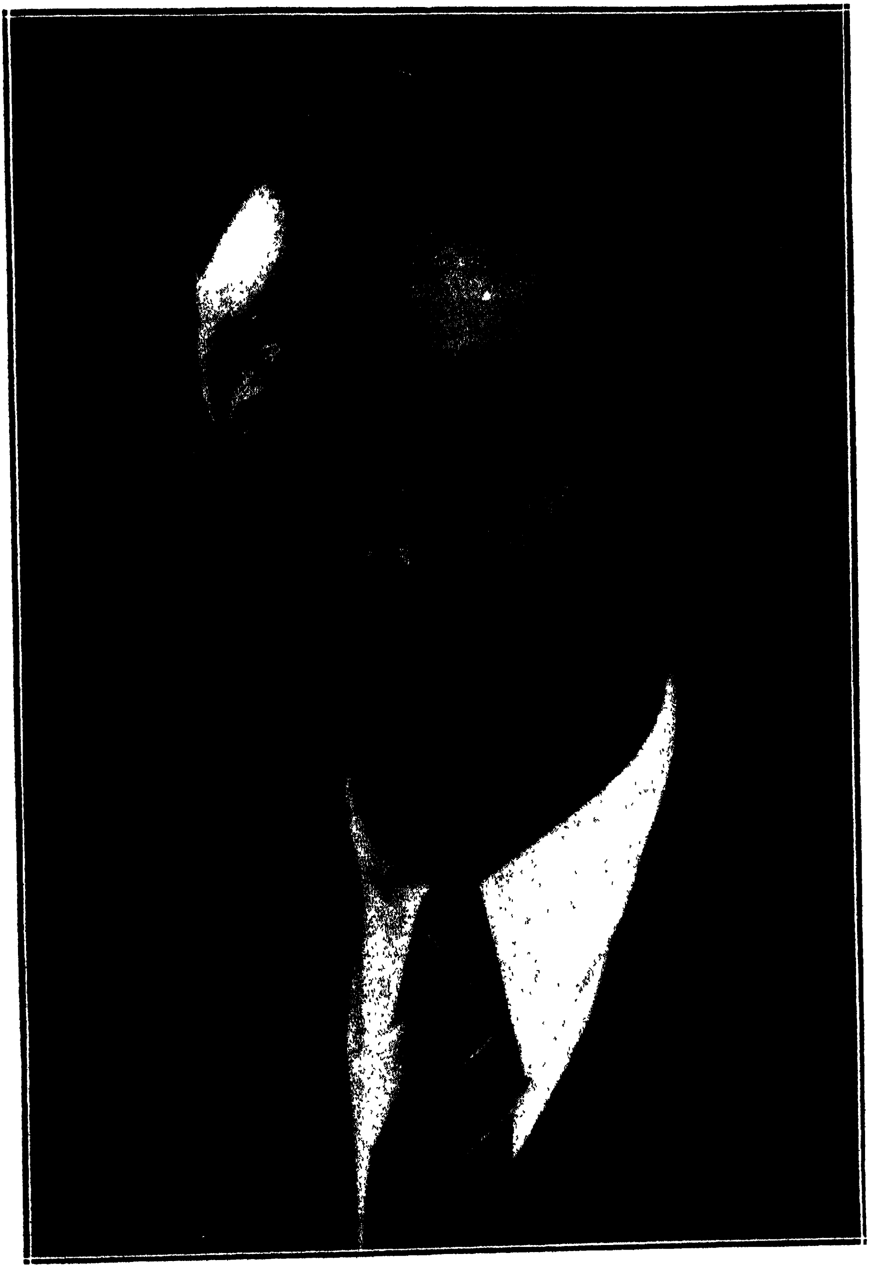
"The recipient of this year's award is now engaged in teaching and research in dairy manufacturing in one of our well known mid-western agriculture colleges. He was graduated from this institution in 1918, received his Master's degree in 1919 and his Doctor's degree in 1922 from this same institution. He has made many valuable contributions to dairy science. His contribution to our knowledge of 'Salt Balances in Milk and Dairy Products' and the 'Effect of Metal on Milk Flavors' are outstanding.

"Probably his two greatest contributions are his two dairy text books. His first book was entitled, 'The Theory and Practice of Ice Cream Making' published in 1932 and revised in 1935. In 1938 his book entitled, 'Market Milk and Related Products' was published. These two books are widely used as text books in this country.

"It was the unanimous opinion of the committee that Doctor Hugo Henry Sommer, Professor, Dairy Industry in the University of Wisconsin, be selected for the 1942 Borden Dairy Manufacturers Research Award."



H. H. SOMMER



W. E. PETERSEN

Mr. Sommer came to the platform and Mr. Wentworth of the Borden Company presented him a gold medal and a check for $1,000.00.

Mr. Anthony, the toastmaster, then introduced Mr. O. E. Reed, Acting Chairman of the Borden Award Committee for Production. Mr. Reed then made the following statement:

"Dr. William Earl Petersen was born February 3, 1892, at Pine City, Minnesota. He received his B.S. Degree at the University of Minnesota in 1916, his M.S. degree at that institution in 1917, and his Ph.D. degree at the University of Minnesota in 1928. He was extension dairy specialist at Kansas State College from 1917 to 1920, field secretary for the Minnesota Holstein Breeders Association 1920 to 1921, and has been a member of the dairy husbandry staff at the University of Minnesota from 1921 to the present time where he holds the position of Professor of Dairy Husbandry.

"While Dr. Petersen's chief interest is the physiology of dairy cattle, his researches have extended into the fields of dairy chemistry and dairy cattle breeding and nutrition.

"His most outstanding work has been in the field of the physiology of milk secretion in which his publications include studies relating to the carbohydrate, fat, protein and mineral metabolism of the mammary gland, and also, hormonal, histological, physical and physical-chemical studies relating to milk secretion. The development of the perfusion apparatus, consisting of an artificial heart and lung for the perfusion of excised mammary glands free of the influence of other organs, has been one of his most important recent contributions.

"In addition, his accomplishments include the development of the Minnesota Babcock reagent, widely used in both buttermilk and blood fat determinations and the development of a practical method of evaluating feeds, adopted by Morrison in his latest edition of 'Feeds and Feeding.' His publications also include the widely used book 'Dairy Science' and two secondary school textbooks: 'American Farming and Agriculture,' Volumes I and II.

"Dr. Petersen has continued to grow in his work from year to year and to expand his great capacity for work in varied fields of investigation. He has remained a student and at the present time in addition to carrying on his teaching, researches and other work, attends no fewer than three different physiological seminars each week during the school year. Perhaps his greatest attribute in his unbounded enthusiasm in research work and his ability to instill this enthusiasm in his students with whom he works very closely, frequently remaining in the laboratory until long after midnight.

"The three members of the Production Award Committee have unanimously selected the recipient for the 1942 Borden Award."

Mr. Wentworth of the Borden Company then presented Mr. Petersen the gold medal and a check for $1,000.00.

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THE USE OF ENZYME-CONVERTED CORN SIRUP IN THE MANUFACTURE OF BULK SWEETENED CONDENSED MILK[1]

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The use of sucrose sugar as a preservative in condensed milk has been practiced for a good many years. However, sucrose shortages that have recently developed make it advisable to consider other types of sweetening agents for preserving food products. In the period immediately following the first World War considerable progress was made in the development of methods for manufacturing refined dextrose, and in 1930 the United States Secretary of Agriculture ruled that the use of pure refined corn sugar in the preparation of an article of food need not be declared upon the label. Ramsey, Tracy and Ruehe (1), in 1933, reported that dextrose could be used to replace one-half the sucrose used in the manufacture of sweetened condensed skim milk. When all the sucrose was replaced with dextrose, mass crystallization took place. Greater tendency toward physical thickening and brown discoloration occurred when the dextrose was used, making special preheating procedures and storage conditions advisable.

The use of corn sirup in the manufacture of condensed milk has not been given serious consideration because of the low dextrose and high dextrin content of this product. Recently, however, high-conversion types of corn sirup have been developed which contain less dextrin and a much higher dextrose equivalent than the ordinary glucose sirup. This is shown by the following comparison of the analysis[2] of representative samples of these two types of corn sirup:

	High-(enzyme) converted corn sirup	Confectioners' type of corn sirup
	%	%
Moisture	18.0	19.7
Dextrose	33.0	17.6
Maltose	23.5	16.6
High sugars	6.4	16.2
Dextrins	18.8	29.6
Ash	0.3	0.3

Received for publication April 11, 1942.

[1] A portion of the thesis submitted by the junior author to the Graduate School of the University of Illinois in partial fulfillment of the requirements for the M.S. degree in Dairy Husbandry.

[2] Data supplied by the laboratory of the A. E. Staley Manufacturing Co., Decatur, Ill.

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A study was planned in which both sweetened whole and sweetened condensed skim milk were manufactured in order to study the practicability of the use of the enzyme-converted corn sirup in the manufacture of sweetened condensed milk. The present report has to do with the development of manufacturing methods. The bacteriological aspects of this study will be reported in a later publication.

METHOD OF STUDY

The batches of sweetened condensed milk were prepared in a commercial sized (36-inch) vacuum pan. The substitution of the sucrose for the high conversion corn sirup was on a dry matter basis.

Pasteurized milk was preheated in an open type hot well with live steam. The sugar was added in the first series in the hot well before preheating, but in the last two series the milk and sugar solutions were preheated separately. The condensing process was continued until the finished product contained approximately 28 per cent milk solids, 42 per cent sugar and 70 per cent total solids in the case of the sweetened skim milk. The sweetened condensed whole milk was standardized to contain 8 per cent fat, 20 per cent serum solids, and 42 per cent sugar solids.

The important variables that were introduced were 1) preheating temperature; 2) method of adding the sugar, 3) amounts and kinds of sugar added; and 4) the temperature and time of storage. These variables were used as shown in table 1.

In order to study the effect of time and temperature during storage, a number of one-half pint glass milk bottles were filled from the batches of sweetened condensed skim milk designated in the table. The bottles were disc capped, sealed with hood caps, and divided into three groups. The first group was stored at 90° F.; the second group was stored at 66° F.; and the third group was stored at 40° F.

Samples stored thus were removed at definite intervals of time and examined for color, body (viscosity), flavor and acidity, and pH.

Color determinations were first made with a K & E color analyzer manufactured by the Keuffel & Esser Company of Hoboken, New Jersey. Due to physical changes in the condensed milk during storage at the high temperature, this method showed some inconsistency in results. Therefore, it was discontinued after four weeks. Thereafter the samples were grouped according to their color as determined visually by the judges.

The viscosity or body was determined by two methods. One method was to use a pipette with the small end removed and calibrated to deliver 10 ml. Two marks were placed on the pipette with a capacity of 5 ml. between the two marks. By the use of a stopwatch the rate of flow from one mark to the other was determined, the temperature being constant at 100° F. Here again difficulty was encountered because of the high viscosity that some of

TABLE 1

Composition and heat treatment given to experimental batches

Batch No.	Sucrose	High conversion corn sirup	Preheating temperature
Series I. Prepared March 31–April 12, 1939. Sugar preheated with the skim milk			
	%	%	°*F.*
A–1	42.0	0.0	170
A–2	42.0	0.0	185
A–3	42.0	0.0	200
B–1	0.0	42.0	170
C–1	21.0	21.0	170
C–2	21.0	21.0	185
C–3	21.0	21.0	200
D–1	10.5	31.5	170
D–2	10.5	31.5	185
D–3	10.5	31.5	200
Series II. Prepared June 3–June 10, 1939. Sugar and skim milk preheated separately			
	%	%	°*F.*
B–1x	0.0	42.0	170
B–2x	0.0	42.0	185
C–1x	21.0	21.0	170
C–2x	21.0	21.0	185
D–1x	10.5	31.5	170
D–2x	10.5	31.5	185
Series III. Prepared September 20–October 16, 1939. Sugar and skim milk preheated separately			
	%	%	°*F.*
A–1x	42.0	0.0	170
B–1x	0.0	42.0	170
C–1x	21.0	21.0	170
C–1z	21.0	*	170
C–1xW†	21.0	21.0	170

40—samples stored at 40° F.
66—samples stored at 66° F.
90—samples stored at 90° F.
* 21% dextrose.
† W = Whole milk.

the samples soon developed. The second method for determining the viscosity was the "stirring test." Wooden sticks were placed in the samples and the resistance to stirring compared. An arbitrary valuation of 3 to 15 was used for recording the relative viscosities. This method checked very well with the other method described, and it was most useful after the samples became too thick to flow.

Flavors were judged in the same manner as market milk. Scores were designated for each sample by the judges.

The titratable acidity was measured by titrating 18 grams (17.6 ml.) of sample with tenth-normal NaOH using phenolphthalein indicator. The samples were prepared by making a 1 to 10 dilution of the sweetened condensed milk by weighing 11 grams into a 99-ml. water blank.

TABLE 2

Color changes of stored samples of condensed milk. Series I. Sugar and skim milk preheated together

Relation of preheating temperature, type of sugar used and storage temperature to the color of sweetened condensed skim milk (Samples placed in order of decreasing color each week).

Number of weeks stored

0	1	2	3	4	5	7	10	11
				Group 1. Color—brown				
D–3–40*	D–3–90 C–3–90 D–3–66 D–3–40 C–3–66	D–3–90 C–3–90 D–3–66 D–3–40 C–3–66	D–3–90 C–3–90 D–3–66 C–3–66 D–3–40 D–2–90 B–1–90 D–1–90	D–3–90 C–3–90 B–1–90 D–2–90 D–1–90 D–3–66 D–3–40 C–3–66 C–2–90	D–3–90 C–3–90 B–1–90 D–2–90 D–1–90 D–3–66 C–2–90 C–1–90 D–3–40 C–3–66	D–3–90 C–3–90 B–1–90 D–2–90 D–1–90 D–3–66 C–2–90 C–1–90 D–3–40 C–3–66	D–3–90 C–3–90 B–1–90 D–2–90 D–1–90 C–2–90 C–1–90 D–3–66 D–3–40 C–3–66	D–3–90 C–3–90 B–1–90 D–2–90 D–1–90 C–2–90 C–1–90 D–3–66 D–3–40 C–3–66
				Group 2. Color—light brown				
C–3–40	C–3–40 D–2–90 B–1–90 D–1–90	C–3–40 D–2–90 B–1–90 D–1–90	C–3–40 C–2–90 D–2–66 B–1–66 C–1–90	C–3–40 D–2–66 B–1–66 C–1–90	C–3–40 D–2–66 B–1–66 D–1–66	C–3–40 D–2–66 B–1–66 D–1–66	C–3–40 D–2–66 B–1–66 D–1–66	C–3–40 D–2–66 B–1–66 D–1–66
				Group 3. Color—light tan				
D–2–40	C–2–90 D–2–66 B–1–66 C–1–90 C–2–66 D–2–40	C–2–90 D–2–66 B–1–66 C–1–90 D–2–40	D–2–40 B–1–40 D–1–66 C–2–66 C–1–66 D–1–40	D–2–40 B–1–40 D–1–66 D–2–66 C–1–66 D–1–40	C–2–66 D–2–40 B–1–40 C–1–66	C–2–66 D–2–40 B–1–40 C–1–66	C–2–66 D–2–40 B–1–40 C–1–66	C–2–66 D–2–40 B–1–40 C–1–66
				Group 4. Color—slight tan				
B–1–40 D–1–40 C–2–40	B–1–40 D–1–66 A–3–90 C–1–66 D–1–40 C–2–40	B–1–40 D–1–66 C–2–66 C–1–66 D–1–40 A–3–90 C–2–40	C–2–40 A–1–90 A–3–90 A–3–66 A–2–90 A–3–40 A–1–66	C–2–40 A–3–90 A–1–90 A–3–66 A–2–90 C–1–40 A–1–66	A–3–90 D–1–40 C–2–40 A–1–90 A–3–66 A–2–90 C–1–40 A–1–66	A–3–90 D–1–40 C–2–40 A–1–90 A–3–66 A–2–90 C–1–40 A–1–66	A–3–90 D–1–40 C–2–40 A–1–90 A–3–66 A–2–90 C–1–40 A–1–66	A–3–90 D–1–40 C–2–40 A–1–90 A–3–66 A–2–90 C–1–40 A–1–66
				Group 5. Color—normal				
C–1–40 A–3–40 A–2–40 A–1–40	A–3–66 C–1–40 A–2–90 A–3–40 A–1–90 A–2–66 A–2–40 A–1–66 A–1–40	A–3–66 C–1–40 A–2–90 A–3–40 A–1–90 A–2–66 A–2–40 A–1–66 A–1–40	A–2–66 C–1–40 A–2–40 A–1–40	A–2–66 A–3–40 A–2–40 A–1–40	A–3–40 A–2–66 A–2–40 A–1–40	A–3–40 A–2–66 A–2–40 A–1–40	A–3–40 A–2–66 A–2–40 A–1–40	A–3–40 A–2–66 A–2–40 A–1–40

* See key on page 767 for history of samples (table 1).

A portable Leeds & Northrup quinhydrone potentiometer was used for measuring the hydrogen-ion concentration of the samples of sweetened condensed skim milk.

EXPERIMENTAL RESULTS

In view of the large amount of data accumulated during the course of this study the experimental results to a great extent are presented in a summarized form.

Effect of Corn Sirup upon Color of Condensed Milk. As previously indicated, Ramsey *et al.* (1) found that when corn sugar was used to replace a part of the sucrose in sweetened condensed skim milk, brown discoloration took place more rapidly than when sucrose alone was used. Although the high-conversion type of corn sirup used in this study contained only about 35 per cent as much dextrose as is contained in corn sugar, it is to be expected that its use would have much the same effect upon color as does corn sugar.

Ramsey *et al.* have shown that there are several factors which may influence the degree of discoloration. The data secured in this study confirm the results of these investigators (tables 2 and 3). The darkest color occurred in the samples from batches preheated at the highest forewarming tempera-

TABLE 3

Effect of variation in time and temperature of storage upon color of sweetened condensed skim milk

(All samples contained 31.5% enzyme-converted corn sirup and 10.5% sucrose)

Order of Color*	Temperature and Time of Storage			
	57° F. Weeks†	60° F. Weeks†	63° F. Weeks†	66° F. Weeks†
1	7.0	3.5	0.5	
2	7.5	4.0	1.0	0.5
3	8.0	4.5	1.5	1.0
4		5.0	2.0	1.5
5	9.0	5.5	2.5	2.0
6		6.0	3.0	2.5
7	10.0	6.5	3.5	3.0
8		7.0	4.0	3.5
9		7.5	4.5	4.0
10		8.0	5.0	4.5
11			5.5	4.5
12		9.0	6.0	5.5
13			6.5	6.0
14		10.0	7.0	6.5
15			7.5	7.0
16			8.0	7.5
17				8.0
18			9.0	
19				9.0
20			10.0	
21				10.0

* Placed in order of increasing color beginning with the first sample showing discoloration at each temperature of storage. All samples on the same horizontal line had the same degree of discoloration.

† Weeks of storage.

ture. Samples containing high-conversion corn sirup developed more color during storage than did the all-sucrose samples. In all cases the changes in color were found to be greatest in the samples stored at 90° F. When the two factors (high preheating temperature and the use of high-conversion corn sirup) were combined, the condensed skim milk was dark enough in color, even when freshly made, to be detectable by eyesight.

The effect of preheating upon color was greater when the temperature was raised from 185° to 200° F. than when raised from 170° to 185° F. In other words, the critical preheating temperature as far as color change is concerned lies in the zone between 185° and 200° F. In the same way the importance of the storage temperature became significantly greater as the temperature was raised from 66° to 90° F.

Although increasing the amount of sucrose replacement with high-conversion corn sirup from 50 to 75 and 100 per cent resulted in greater tendencies towards discoloration, the differences were not marked.

These data indicate that when high-conversion corn sirup is used in the manufacture of sweetened condensed skim milk, both the preheating temperature and the temperature of storage must be properly controlled if the product is to be kept for several weeks before using.

The importance of the temperature of preheating and the storage temperature was found to decrease considerably when the milk and sugar were preheated separately. For example, when the milk and sugar were preheated together, the sample containing 42 per cent high-conversion corn sirup (B–1–66) preheated at 170° F. and stored at 66° F. for 3 weeks had a noticeable creamy color. However, when the milk and sirup were preheated separately, the sample containing 42 per cent high-conversion corn sirup (B–1x–66) preheated at 170° F. and stored at 66° F. was only slightly discolored after 12 weeks of storage. It was also observed that when the milk and sirup were heated separately, samples stored at 90° F. were the only samples considered off color after three months' storage, while in the case of the batches in which the skim milk and sirup were preheated together, some of the samples stored at 66° F. for three months became sufficiently discolored to be considered off color.

The results also show the influence of storage temperature when different types of sugar were used. Samples containing a sucrose replacement of 50 per cent dextrose, 50 per cent high-conversion corn sirup and 100 per cent high-conversion corn sirup were discolored sufficiently at the end of four weeks' storage at 90° F. to be considered off color. Even the samples containing all sucrose became highly discolored during the third month of storage.

The use of whole milk rather than skim milk did not materially effect the development of discoloration.

Table 3 shows the relation of time and temperature of storage to the

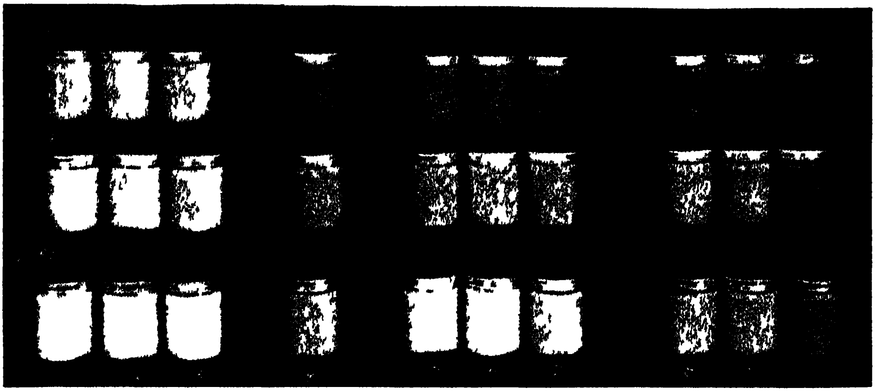

Fig 1 Samples of sweetened condensed skim milk after storage at 40°, 66° and 90° F for 11 weeks Samples 1, 2, 3 contained sucrose Samples 4 contained Sweetose. Samples 5, 6, 7 contained Sweetose (50% replacement). Samples 8, 9, 10 contained Sweetose (75% replacement) Samples 1, 4, 5, 8—forewarming temperature—170° F. Samples 2, 6, 9—forewarming temperature—185° F Samples 3, 7, 10—forewarming temperature—200° F. In all cases milk and sugar were heated together.

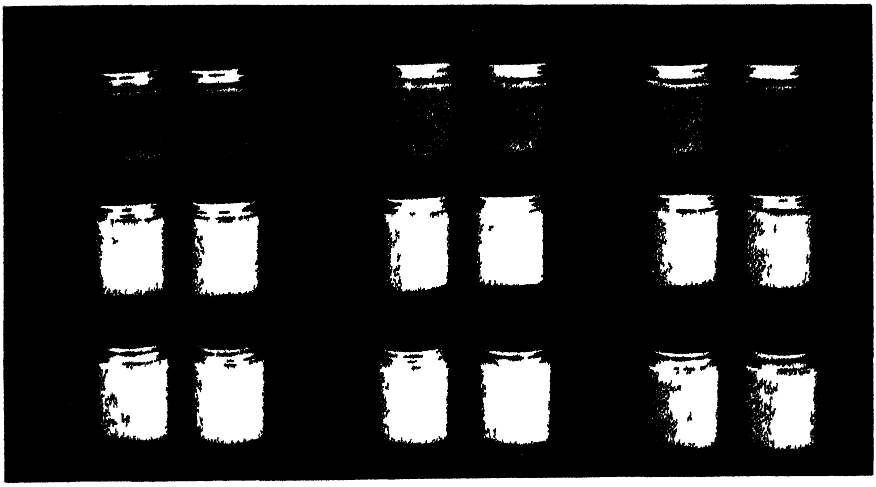

Fig. 2. Samples of sweetened condensed skim milk containing different amounts of Sweetose (50, 75 and 100% replacement) after storage for 3 months at 40°, 66° and 90° F. Samples Number 1—forewarming temperature—170° F Samples Number 2—forewarming temperature—185° F. In all cases milk and Sweetose sirup were heated separately.

TABLE 4

*Factors related to progressive thickening of sweetened condensed milk. Series 1. Sugar and skim milk heated together. Relation of preheating temperature, amount and type of sugar used, and storage temperature to the body of the sweetened condensed skim milk**

	A–1†	A–2	A–3	B–1	C–1	C–2	C–3	D–1	D–2	D–3
Stored at 40° F.										
Original	3.00	5.00	7.00	5.50	4.00	6.50	8.00	4.00	6.00	7.00
1 week	3.00	5.00	7.00	5.50	4.00	6.50	8.00	4.50	6.50	7.00
2 "	3.00	5.00	7.00	5.50	4.00	6.75	8.50	4.50	6.50	8.00
3 "	3.00	5.00	7.00	5.50	4.00	6.75	8.50	5.00	6.50	8.00
4 "	3.00	5.00	7.00	5.50	4.00	6.75	8.50	5.00	6.25	8.00
5 "	3.00	5.00	7.00	5.50	4.00	6.75	8.50	5.00	6.25	8.00
7 "	3.00	5.50	7.00	6.00	4.00	7.00	9.00	5.00	6.25	8.00
10 "	3.00	5.50	7.00	6.00	4.75	7.00	10.00	5.00	6.25	8.00
11 "	4.00	6.00	7.00	6.00	5.00	7.50	10.50	5.25	7.00	9.25
Stored at 66° F.										
1 week	3.50	5.50	8.50	6.50	5.00	7.25	9.25	5.50	7.50	8.75
2 "	4.50	5.75	8.50	6.50	5.00	7.50	10.00	5.50	8.25	9.50
3 "	4.50	6.50	9.00	7.00	5.25	8.00	10.75	6.00	8.25	10.25
4 "	5.00	7.00	10.00	7.50	5.50	8.25	11.25	6.50	9.50	11.00
5 "	5.25	7.25	10.00	8.25	5.75	8.50	12.00	7.00	9.75	11.50
7 "	6.00	7.75	10.50	9.00	6.25	9.25	12.25	7.50	10.50	11.75
10 "	6.25	8.00	10.50	9.50	6.50	10.00	13.00	8.00	11.00	12.25
11 "	6.75	8.75	11.00	10.50	7.00	11.00	14.00	9.25	11.25	12.50
Stored at 90° F.										
1 week	4.00	6.50	9.00	7.50	6.00	8.00	9.75	6.50	8.50	9.25
2 "	5.00	7.25	9.00	9.75	7.25	10.00	11.00	8.25	10.50	10.50
3 "	6.00	8.00	9.50	10.50	7.00	10.75	12.00	9.50	11.00	11.25
4 "	7.00	8.00	10.00	11.00	7.50	11.25	12.50	10.50	12.00	12.25
5 "	7.50	9.50	11.00	11.75	8.50	12.25	13.00	11.00	12.25	12.75
7 "	8.00	10.00	11.25	12.00	8.75	12.50	13.25	11.25	12.50	13.00
10 "	8.25	10.50	11.75	12.50	10.00	13.25	14.00	12.00	13.25	13.50
11 "	9.00	11.50	12.50	13.00	11.00	13.50	14.50	13.00	13.50	13.75

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* The higher the viscosity value the more plastic the mass.

† See key to data on page 767 for history of samples (table 1).

degree of discoloration. Samples of sweetened condensed skim milk containing 10.5 per cent sucrose and 31.5 per cent high-conversion corn sirup were placed in test tubes and incubated at 57°, 60°, 63°, and 66° F. The temperatures of the incubators did not vary more than ± 0.5°. The data show that when a storage temperature of 57° F. was used, it was not until the seventh week that a perceptible change in color occurred. When the samples were stored at 60° F. a change in color was noted after 3.5 weeks, while at 63° F. and 66° F. there was a change in color of the samples after ½ week. It should be noted that the samples stored at 57° F. for 9 weeks had the same degree of discoloration as those stored at 60° F. for 5.5 weeks, 63° F. for 2.5 weeks, and 66° F. for 2 weeks. The discoloration of samples stored at 66° F. for 10 weeks, however, was not sufficient to make the condensed milk unsalable.

Progressive Thickening. The relation of the preheating temperature, the type of sugar used, and the storage temperature to progressive thickening was determined. Preheating temperature had a noticeable effect, the highest temperature causing the greatest increase in viscosity. The all-sucrose samples exhibited less change in body during storage than did any of the samples containing high-conversion corn sirup (table 4). All the samples stored at 40° F. increased in viscosity very slowly, many remaining almost the same for the entire storage period of 11 weeks. However, when the samples were stored at 66° F. the viscosity increased rapidly, particularly in the case of the samples from batches preheated at 200° F. The samples stored at 90° F. thickened most rapidly.

Data from series II show the relation of preheating and storage temperatures to the progressive thickening of sweetened condensed skim milk containing varying amounts of high-conversion corn sirup when the skim milk and sugar were preheated separately. Both factors—preheating temperature and storage temperature—were found to have less effect on the body when the skim milk and sugar were preheated separately than when heated together.

The relation of storage temperature to the progressive thickening of sweetened condensed skim milk samples containing three different types of sugars was also determined (Series III). In this series the sugar (or sirup) and milk were preheated separately at 170° F. The all-sucrose samples were found to be the least affected by progressive thickening. The dextrose samples ranked next to the all-sucrose samples in this respect. The samples containing high-conversion corn sirup were more viscous than either the dextrose or sucrose samples. It is possible that the higher viscosity of the samples containing high-conversion corn sirup was partly due to the dextrin present in the corn sirup.

In the third series, the data also show that the sweetened condensed milk prepared from whole milk thickened less than did the skim milk product.

TABLE 5

Changes in hydrogen ion concentration in stored condensed milk. Series I. Sugar and skim milk preheated together. Relation of storage temperature to changes in pH of sweetened condensed skim milk*

	A-1†	A-2	A-3	B-1	C-1	C-2	C-3	D-1	D-2	D-3
Samples stored at 40° F.										
Original	6.07	6.10	6.12	5.96	6.07	6.10	6.07	6.10	5.93	5.96
4 weeks	6.08	6.21	6.25	5.94	6.04	6.06	6.04	6.06	6.08	6.08
7 "	6.05	6.17	6.22	Broken	6.00	6.02	5.99	6.02	6.00	6.04
11 "	6.12	6.26	6.33	5.95	6.09	6.11	6.07	6.07	6.07	6.17
Ave.	6.08	6.185	6.23	5.95	6.05	6.048	6.04	6.06	6.02	6.06
Samples stored at 66° F.										
4 weeks	6.28	6.31	6.35	6.04	6.21	6.21	6.14	6.19	6.19	6.23
7 "	6.21	6.26	6.31	6.02	6.19	6.21	6.14	6.16	6.17	6.19
11 "	6.31	6.36	6.39	6.12	6.24	6.26	6.19	6.21	6.23	6.24
Ave.	6.26	6.31	6.35	6.06	6.21	6.22	6.15	6.18	6.19	6.22
Samples stored at 90° F										
4 weeks	6.31	6.34	6.38	6.05	6.22	6.21	6.14	6.21	6.21	6.21
7 "	6.29	6.27	6.34	6.07	6.21	6.17	6.12	6.14	6.16	6.16
11 "	6.45	6.46	6.48	6.19	6.33	6.33	6.28	6.29	6.31	6.28
Ave.	6.35	6.35	6.40	6.10	6.25	6.23	6.18	6.213	6.22	6.216

* pH determined on undiluted samples.

† See key to data on page 767 for history of samples (table 1).

The greatest difference in the bodies of these two products occurred in those samples stored at 90° F.

One interesting observation noted in the third series was the greater degree of progressive thickening of the samples containing 50 per cent of the sucrose replaced with high-conversion corn sirup as compared to either the 75 per cent or 100 per cent high-conversion corn sirup replacement. This was true regardless of the preheating temperature.

Flavor. There were some changes in flavor scores of all the samples during the storage period. In series I the least change occurred in the samples stored at 40° F., while the greatest changes in flavor were in those samples stored at 90° F. The higher the preheating temperatures the lower the initial scores and the fewer changes there were in the flavors during storage. The samples containing high-conversion corn sirup likewise showed less reduction in flavor scores than did the sucrose samples, although the initial scores of the all-sucrose samples were higher. The high-conversion corn sirup samples were scored down somewhat because of a slight bitterness due in part, probably, to the lesser degree of sweetness.

In series II the relation of preheating temperature and storage temperature to the flavor of sweetened condensed skim milk containing 50, 75, and 100 per cent high-conversion corn sirup replacements was found to be essentially the same as in series I. However, when the skim milk and sugar were heated separately as in the second series, there was less change in flavor scores during storage. These data indicate that the best flavor can be obtained by preheating the skim milk and sugar separately.

Samples of sweetened condensed skim milk containing different types of sugars after being stored for three months ranked in flavor in descending order as follows: 1) 50 per cent of the sucrose replaced with dextrose, 2) 50 per cent of the sucrose replaced with high-conversion corn sirup, 3) 42 per cent high-conversion corn sirup, and 4) 42 per cent sucrose. This ranking was the same regardless of the temperature of storage. In fairness to the all-sucrose sample it should be mentioned that this sample was somewhat abnormal due to a separation of lactose crystals.

After four weeks of storage the flavor of the sweetened condensed milk made from skim milk was found to be much better than that of the product made from whole milk. The reason for the lower flavor score of the sweetened condensed whole milk product was the development of an oxidized flavor.

pH of Sweetened Condensed Milk. When the sugar and skim milk were preheated together the samples containing all-sucrose had a higher pH when the preheating temperature, storage temperature, and storage period were increased (table 5). The samples containing all-sucrose had a higher pH than the samples in which a part or all of the sucrose had been replaced with high-conversion corn sirup. This effect of the high-conversion corn sirup is to be expected since the sirup has a pH of 4.8 to 5.0.

Samples containing high-conversion corn sirup increased in pH when the storage temperature was increased, but increasing the preheating temperature and storage period did not cause the pH to change to the same degree as in the case of the all-sucrose samples.

When the sugar and skim milk were preheated separately, and when 50, 75, and 100 per cent of the sucrose was replaced with high-conversion corn sirup, increasing the preheating temperature and increasing the storage temperature from 40° F. to 66° F. resulted in an increased pH. On the other hand, the samples stored at 90° F. had a lower pH. Increasing the storage period also caused the pH of the samples to decrease. The decrease was not sufficient, however, to indicate bacterial spoilage of any consequence.

Data from series III show that samples containing all-sucrose had a higher pH than those containing either high-conversion corn sirup or dextrose.

Sweetened condensed milk samples made from whole milk had a higher pH than the samples made from skim milk. This is to be expected because of the higher serum solids content of the latter product.

SUMMARY

The use of enzyme-converted corn sirup to the extent of 50 to 100 per cent replacement of the sucrose in the manufacture of bulk sweetened condensed skimmed and whole milk has been found practicable. Best results from the standpoint of color and physical thickening were obtained when the milk and sirup were heated separately and when the preheating temperature was limited to 185° F. There was less physical thickening and less brown discoloration when the samples were stored at low temperatures. For commercial operations the storage temperature need not be lower than 60° F. unless the condensed milk is to be held for more than three months. At 90° F. the change in color and body takes place rapidly. Judging from the flavors and pH measurements, samples of condensed milks sweetened with high-conversion corn sirup (50 to 100 per cent replacement) have normal keeping qualities.

The procedure recommended for the use of high-conversion corn sirup is as follows:

1. Replace 50 to 100 per cent of the sucrose with the corn sirup.
2. Preheat milk to 185° F.
3. If a minimum viscosity and color effect is desired, preheat milk and sirup separately, drawing sirup into the vacuum pan near the end of the run.
4. Store the condensed milk at as low temperatures (60° F. or less) as convenient unless it is to be used within a few days after manufacture.

ACKNOWLEDGMENT

The high-conversion corn sirup used in this study was supplied by the A. E. Staley Manufacturing Company, Decatur, Illinois.

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ORTHOPHOSPHORIC ACID AS A CHEESE SOLVENT

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The possibility of using a weak solution of orthophosphoric acid as a solvent for cheese curd in determining extraneous matter in cheese was studied by us. Preliminary trials showed that a boiling 1.0 per cent aqueous solution of phosphoric acid was capable of dissolving Swiss, American, Limburger, cottage, Romano and process cheese, including their rinds.

Observations were made on the action of the 1.0 per cent phosphoric acid solution on some of the cellulose, chitin, and inorganic types of extraneous matter occasionally found in cheese. These types of extraneous matter lost approximately 5 per cent of their weight during 20 minutes of boiling in 1.0 per cent phosphoric acid. Their structures were not visibly impaired.

A large series of extraneous matter determinations on green and aged cheeses shows that 50 grams of cheese dissolves readily in 500 ml. of 1.0 per cent phosphoric acid. It appears likely that 50 grams of cheese would be a satisfactory amount of cheese to use since this would be comparable to the amount of milk solids in the pint of milk used in the milk sediment test. However, if a larger sample needs to be examined for sediment it is only necessary to use a correspondingly larger amount of phosphoric acid solution to dissolve it. The following method was adopted for removing the extraneous matter from cheese for examination and scoring.

APPARATUS

1. One cheese trier
2. One food grater with ⅛″ holes.
3. A 1-liter beaker.
4. One pressure milk sediment tester.
5. Milk sediment pads.
6. Irish poplin cloth pads of the same size as the milk sediment pads.[1]
7. One 500-ml. graduate.
8. One 5-ml. graduate.
9. One glass stirring rod.
10. One hot plate or gas burner and tripod.

REAGENTS

1. An aqueous solution containing 1.0 per cent by weight of orthophosphoric acid. This is made by adding 7 ml. of orthophosphoric acid to 1000 ml. of water.
2. Acetone.[1]

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[1] **Suggested by R. E. Miersch and Dr. W. V. Price, University of Wisconsin Dairy Department, Madison, Wisconsin.**

PREPARATION OF SAMPLE

Take a sample of cheese by plugging the cheese to be examined two or three times with the cheese trier. Grate the cheese plugs on the cheese grater, being careful to exclude all outside contamination.

DETERMINATION

Weigh 50 grams of the grated cheese into a clean 1-liter beaker and add to it 500 ml. of 1.0 per cent phosphoric acid. Heat to boiling, stirring occasionally to prevent cheese from sticking to the bottom of the beaker. Boil until all the cheese is dissolved and filter immediately through the pressure milk sediment tester with the poplin cloth pad on top of the milk sediment pad. Wash the beaker and tester with distilled water, forcing the wash water through the filter pads. Remove the pads carefully and examine the extraneous matter on the surface of the poplin cloth pad. The moisture remaining in the sediment pad can be removed by rinsing the pad with 5 ml. of acetone before it is taken from the sediment tester.

GRAZING HABITS OF DAIRY CATTLE

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Maximum returns from any pasture depend upon cropping practices conducive to growth response of plants, and upon cattle management to avoid waste. The inter-relationship of these two primary factors and the need for proper balance between the two in pasture management are apparent. Combination of rotational grazing with clipping, fertilizing, etc., are typical attempts to consider both major factors. Many conditions influence the system of cattle management adopted, such as short time pasturage daily to make limited pasture go further, night pastures, etc. Grazing habits of cows are of interest from the standpoint of the wellbeing of both the cattle and the pasture.

GRAZING HABITS OF MILKING COWS DURING DAYTIME

Comparative time spent in grazing by milking cows on six different pastures was recorded. Two cows were used on each pasture. The pastures averaged about four acres. Observations were taken for three consecutive days on each pasture, but not concurrently. The observations covered a 12-hour daylight period for the first two days and 11 hours the third day, or an average of 11 hours and 40 minutes per day. Records were kept on the time spent in grazing, number of times each cow lay down, and the number of times each cow drank.

Summarization of results (table 1) showed the cows spent slightly less than half the time in grazing while on good pasture. Four different pairs of cows on four different good pastures averaged practically the same time spent in grazing, the variation being from 46 per cent of the day to 50 per cent, or a maximum difference between the groups of only 20 minutes in approximately five and one-half hours. One pair of cows was pastured on a field of mixed brome grass and alfalfa which was not uniform in stand. It was rated as *fair* pasture because it appeared to have less feed on it than the better pastures. Another field rated as *poor* pasture consisted of short wheat. On the fair pasture the cows spent 56 per cent of the time grazing and on the poor pasture 62 per cent of the time. Compared with an average of 5.6 hours of grazing on the four good pastures, the grazing time on fair pasture was 6.5 hours and 7.3 hours on poor pasture. Thus the cows spent 31 per cent more time grazing on the poor pasture than the average on the good pastures. Whether this difference would have been even more pro-

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TABLE 1

Grazing habits of dairy cows on several pastures—daytime only

No. cows	Kind of pasture			No. in trials		Av. time grazing		Ave. No. drinks	Av. time not grazing		Ave. No. times lying down	Ave. total daily hrs.
	Height inches	Variety	Quality	Days	Cows	Hrs.	Per cent		Hrs.	Per cent		
2	4–15	Rye	Good	3	2	5.4	46	3	6.3	54	6	11.7
2	4–15	Rye	Good	3	2	5.4	46	3	6.3	54	3	11.7
2	4–15	Rye & vetch	Good	3	2	5.7	49	4	6.0	51	3	11.7
4	4–12	Brome & alfalfa	Good	3	4	5.8	50	4	5.9	50	4	11.7
4	4–10	Brome & alfalfa	Fair	3	4	6.5	56	4	5.2	44	2	11.7
2	2– 3	Wheat	Poor	3	2	7.3	62	4	4.4	38	2	11.7

nounced had the cows been on the pastures constantly with no supplementary feed is not known.

While on pasture the cows drank an average of from three to four times during the day with no apparent relationship to quality of pasture. This would indicate the importance of having water readily available in the pastures for milking cows. The cows lay down an average of four times daily on good pastures and only two times daily on fair and poor pastures. This indicates that the cows had more difficulty getting their fill of grass, but whether there is any relationship with the time spent in ruminating in proportion to the amount of feed ingested, or in utilization of feed is unknown.

GRAZING HABITS OF DRY COWS AND HEIFERS CONTINUOUSLY ON PASTURE

A more complete study of grazing habits of dairy cattle was made with a group of 56 dry cows and yearling heifers being pastured on a 30 acre field of Balbo rye (3). The pasture was excellent, the herbage being 6 to 10 inches high. The animals remained on the pasture constantly and received no supplementary feed. The animals were kept under constant watch and the numbers grazing, not grazing, and lying down were recorded at five minute intervals for three consecutive 24-hour periods. A heavy rain for several hours interrupted observations on one day. A period (April 10–14, 1940) was selected when the moon was about half full in order that night counts could be made without distracting the cows with a light.

TABLE 2

Grazing habits of dry cows and heifers continuously on pasture

	Grazing		Standing		Lying down	
	Hrs.	Per cent	Hrs.	Per cent	Hrs.	Per cent
	Twelve-hour day and night					
Daytime	4.3	36	2.8	23	4.9	41
Nighttime	2.7	23	1.2	10	8.1	67
24-hr. ave. total	7.0	29	4.0	17	13.0	54
	Fourteen-hour day and ten-hour night					
Daytime	5.6	40	3.5	25	4.9	35
Nighttime	1.6	16	0.4	4	8.0	80

Summarization of the results (table 2) showed that the animals grazed an average of 7.0 hours, or 29 per cent of a 24-hour period. The animals spent an average of 4.0 hours, or 17 per cent of the time in walking or standing without grazing, and 13.0 hours, or 54 per cent of the 24-hour periods in lying down. When the 24-hour period was divided into two 12-hour periods, 6:00 A.M. to 6:00 P.M. for daytime and the similar hours for night, the time the animals spent in grazing averaged 4.3 hours, or 36 per cent of the day period; and 2.7 hours, or 23 per cent of the night period. Since the sun rose at 6:00 A.M. and set at 7:00 P.M. during the trials, a 14-hour daylight period of from 6:00 A.M. to 8:00 P.M. inclusive would be a more typical daylight feeding period and 8:00 P.M. to 6:00 A.M. would more nearly represent night conditions. Comparisons of the grazing habits of the animals on an average 14-hour day and 10-hour night showed that they grazed 40 per cent of the time in daytime and 16 per cent of the time at night. They spent 25 per cent of the daytime and only 4 per cent of the night walking or standing. The animals were lying down 35 per cent of the daytime and 80 per cent of the night.

The average of 7 hours in 24 spent in grazing is in reasonable agreement with the report of 8 hours found by Johnstone-Wallace (2), with milking cows on different types of pastures. His report that cows spend as much time grazing at night as in the daytime is not supported by the results of this investigation. The time spent in grazing during the day time of this trial (29 per cent) is less than reported for milking cows (46–62 per cent) in table 1, but the differences in pasture, the effect of pasturing during daytime only, and the differences between milking cows and dry cows may account for longer grazing periods in the former trial. The fact that these 56 animals averaged 54 per cent of the 24-hour periods lying down is in agreement with the reports of Fuller (1) who found that cows fed in stanchions during winter averaged 50 per cent of the time lying down. He also reported that the cows spent an average of 5.95 hours eating, or 25 per cent of the 24-hour period; and 8.1 hours ruminating, or 34 per cent of the time. Woodward (4) reported that cows on good pastures consumed a maximum of 150 pounds of grass daily. From these facts it would seem cows on poor pastures not only must spend more energy in obtaining sufficient food but that the normal time spent in ruminating may be involved.

Detailed study (fig. 1) of the grazing habits of the cows showed that the animals tended to have about four primary grazing periods during the daytime. The first period began about 5:30 A.M. and lasted approximately two and one-half hours. Another feeding period began about 10:00 A.M., or slightly before, and lasted from an hour to an hour and a half. The third feeding period began between 12:30 and 1:00 o'clock and continued for about two hours. The fourth daylight grazing period began about 6:00 P.M. and continued until about 8:30 P.M.

The night period was considered between 8:30 P.M. and 5:00 A.M. During the night the cows had two primary feeding periods, one from about 9:30 P.M. to 11:00 P.M. and another from about 1:00 A.M. to 3:30 A.M. Thus, it appeared that the animals as a group tended to fill about six times in 24 hours, four times during daylight and twice during the night. In the daytime the entire herd tended to graze as a complete unit or lie down as a group, but at night the lower peaks in grazing numbers with periods less sharply defined indicated more individual action by the animals.

These observations are of interest in pasture management because the cows tend to graze less in night pastures than in daytime pastures. It is also interesting to note that the herd does not fill before the time when the average herd is brought into the barn for the morning milking. This would indicate that in summer management of cows earlier milking might be desirable if the cows are to get a complete fill after milking before the flies and heat interfere with grazing habits. These data were obtained in April when the climate was ideal for cow comfort. What effect heat and flies during mid-summer days would have on grazing habits is not known.

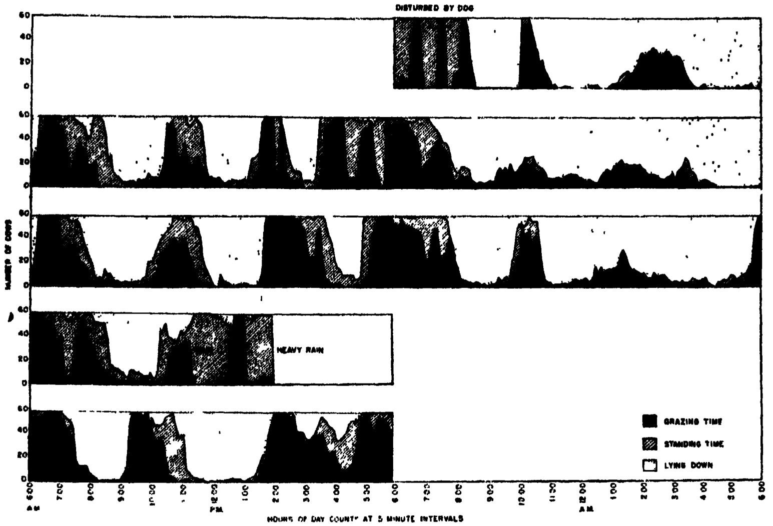

FIG. 1. Observations on 56 dry cows and heifers continuously on pasture, with the number grazing, standing and lying down indicated throughout 24-hour periods.

SUMMARY AND CONCLUSIONS

Comparative time spent in grazing by milking cows on six different pastures during the daytime was recorded. On good pasture the cows spent slightly less than half the time in grazing. On fair pasture the cows spent 55 per cent of the time grazing and on poor pasture 62 per cent of the time. Compared with an average of 5.6 hours of grazing on good pastures, the grazing time on fair pasture was 6.5 hours and 7.3 hours on poor pasture. Thus, the cows spent 31 per cent more time in grazing on poor pasture than on good pasture. The cows drank an average of from three to four times during the day with no apparent relationship to quality of pasture. The cows lay down an average of four times daily on good pasture and only two times daily on fair and poor pasture.

A more detailed study of 56 dairy cattle on Balbo rye pasture constantly with no supplementary feed showed that the animals spent an average of 7 hours grazing, 4 hours standing or walking, and 13 hours lying down during a 24-hour period. Comparison of a daylight period of 14 hours with a night period of 10 hours showed that the animals grazed an average of 40 per cent of the time during the day and 16 per cent during the night. They spent 25 per cent of the time walking or standing during the day, and 4 per cent during the night. The animals were lying down 35 per cent of the daytime and 80 per cent of the night.

The grazing habits of the animals from day to day were quite uniform. They had four primary grazing periods during the day and two during the night, but the night grazing periods were not so pronounced as a group as were those in the daytime.

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FACTORS MODIFYING THE RATE OF FERMENTATION OF RUMEN INGESTA AND THEIR POSSIBLE RELATION TO BLOAT IN DAIRY CATTLE*

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Fermentation of the rumen contents is essential for the normal digestion of fiber-containing feeds. Enormous changes regularly occur in the environment of the bacteria responsible for this fermentation. The ingestion of water in varying amounts and temperature, and the consumption of feed varying from zero to thirty per cent in fiber as well as in sugar content are factors to be dealt with at a moment's notice. Apparent lack of palatability of a feed might simply be an outward indication of depressed fermentation in the rumen as a result of adverse environmental conditions.

Although no extensive study has been made of the organisms responsible for the fermentation in the rumen, many gas producing organisms, such as *Bact. coli*, have been isolated from the rumen contents (5, 15). The optimum pH for growth of *Bact. coli* and many similar organisms is around pH 7.0 or a little above (10, p. 223). Studies have shown that the acidity of the rumen contents varies from pH 5.5 to 7.7 (4, 6, 9, 12). The hourly fluctuations depend largely on: the chemical composition of the feed, the amount of organic acids produced by the bacteria, and the neutralizing effect of the saliva (saliva has a pH of about 8.1) being secreted. Fresh green feeds apparently increase the rate of fermentation and decrease the amount of saliva required for mastication with the result that the pH of the rumen contents drops (6). Dry feeds, especially roughages, result in a higher pH of the rumen contents (15).

Since the optimum temperature for growth by many types of bacteria is between 37° and 45° C. (10, p. 222) it is quite probable that the changes in temperature occurring in the rumen do not seriously modify fermentation. One would expect the ingestion of large quantities of cold water only temporarily to influence the temperature of the rumen.

Dilution of the rumen contents with water affects the osmotic force of the milieu as well as the concentration of the soluble nutrients upon which the bacteria subsist. Dilution of the food supply probably affects bacteria less than dilution of their own metabolic products (10, p. 222). Growing bacteria not only are rapidly consuming oxidizable materials like glucose but they are also eliminating metabolic products which may seriously interfere with their own growth. Some of these decomposition products resulting

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from bacterial fermentation are: acetic, lactic and butyric acids, alcohol, ammonia, indol, skatol, hydrogen sulfide and mercaptans.

Analyses have shown that carbon dioxide and methane are the principal gases resulting from fermentation in the rumen (1, 3, 7, 11, 13) although hydrogen, hydrogen sulfide, carbon monoxide and other gases have been identified. Nitrogen and oxygen are also present as a contamination from the outside air. Washburn and Brody (13) found that the per cent of CO_2 increased rapidly after feeding. On a grass diet the peak was reached much more quickly than when alfalfa hay or alfalfa hay and grain were fed. The relative amount of CO_2 in the rumen gas varied from 80 per cent shortly after grass was fed to 10 per cent, twenty-three hours later. The per cent of CH_4 for the same periods were 20 and 9 respectively. Contamination with air apparently caused these marked decreases as the percentage of nitrogen and oxygen present rose from zero in both cases to 68 and 13 per cent respectively. (Analyses were for CO_2, CH_4, O_2, H_2, and N_2 only.) By measuring the amount of methane in the expired air and calculating from the above ratios the per cent of CO_2 due to fermentation, it was found that a dry cow shortly after being fed 4.5 kilos of grass (dry matter basis) produced carbon dioxide and methane at the rate of 32 and 10 liters, respectively, per hour. Five hours later the rates had dropped to 13 and 6 liters per hour and 22 hours later the rates were 1.5 and 1.2 liters per hour. It is interesting to find that on dry hay or on hay and grain the rate of methane production was almost the same as the figures just cited although the dry matter intake was only half as large. However, the rate of production of carbon dioxide was about two-thirds as large under these conditions. Data on lactating cows show a maximum rate of production of methane of 16 liters per hour shortly after feeding (for the same sized animal—about 900 lbs.). The feed intake was not given.

Although the amount of oxygen present in the gas in the rumen is relatively small (zero to three per cent) the majority of bacteria (like *Bact. coli*) are facultative or obligatory anaerobes and do not require free oxygen. The solubility of oxygen in water is so low that, were it not for the constant agitation of the rumen contents and the fact that much of the contents are out of water a large part of the time, it would appear improbable that strictly aerobic bacteria would even survive and grow in the rumen.

A wide variety of products serve as a source of feed for the bacteria in the rumen because bacteria secrete innumerable kinds of enzymes (2). Glucose and ammonium chloride are about the simplest sources of carbon and nitrogen for bacteria. Since the cow's ration doubtless always contains thousands of different compounds, either real or potential, it is not surprising if the usual changes in diet which take place on the farm only moderately influence bacterial fermentation in the paunch.

Chief among the substances normally providing energy for anaerobic life are carbohydrates. Sugars, especially glucose, greatly increase growth under anaerobic conditions (10, p. 52, 199). It should also be noted that anaerobic fermentation is exothermic and doubtless helps provide the heat for warming the water or other cold feed ingested by the cow.

When considerable fermentation does occur, as indicated by excessive gas production, we speak of it as bloat or tympanites. This rarely occurs except when the cow is turned onto legume pasture (7, 8, 14). Although many analyses have been run on the principal constituents of legumes and non-legumes, no essential product for bacterial metabolism has ever been isolated from legumes which is not present in non-legumes. Hence there is no ready clue for determining why cows frequently bloat on legume pasture and not on non-legume pasture.

The experiments reported in this study were carried out *in vitro* on rumen ingesta obtained from a three-year-old Jersey cow with a rumen fistula. At first the cow received a ration containing a grain mixture, alfalfa hay and corn silage. The silage was later omitted. In the spring, bluegrass pasture was substituted for the alfalfa hay.

In every case the rumen ingesta were removed just previous to their use. Since the experimental procedure required 1200 grams of ingesta, a somewhat larger quantity was removed and thoroughly mixed before being placed in the fermentation apparatus. Because conditions in the rumen are constantly changing, control samples were included with each trial. In the tables which are presented, "Experimental Sample No. 1" should be compared with "Check Sample No. 1," *et cetera.*

The apparatus for gas-volume study consisted of six units. The ingesta for each unit were placed in a 500-cc. Erlenmeyer flask, which was in turn connected with a gas-collecting tube. Two hundred grams of ingesta were placed in each flask unless otherwise noted. The gas-collecting apparatus consisted of 6 pyrex tubes 48 mm. in diameter and one meter in length. These tubes were filled with a solution containing 235 gm. of NaCl and 5 cc. of lactic acid per liter of water. Previous to each experiment, carbon dioxide was bubbled through the solution to insure saturation.

The water bath in which the samples of ingesta were fermented was held at 38.5° C. except for studies of the influence of temperature on fermentation. Gas volume readings were taken at intervals of 1, 2, 3, 4, 5 and 20 hours after each trial was started. By using a leveling bulb direct readings could be made from the gas-collecting tubes. No corrections were made for changes in temperature because check and experimental tubes were exposed to identical conditions. However, an effort was always made to keep the room temperature constant.

The hydrogen-ion concentrations of the materials under study were determined electrometrically, using a quinhydrone electrode. The readings were usually taken both before and after fermentation so that changes due to treatment could be noted.

RESULTS

The data in table 1 (see also fig. 1) serve to illustrate the general trend in rate of gas formation when rumen ingesta are fermented *in vitro*. The results obtained with the two rations show the reduction in rate of fermentation due to the inclusion of silage in the ration. The cause of this slower rate of fermentation is not definitely known although acetic or lactic acid has the same effect when added to silage-free ingesta. Preliminary trials indicated that small amounts of lactic acid actually stimulated fermentation. But with acetic acid, fermentation was only about one-tenth of normal when as little as two per cent of acetic acid was mixed with the rumen contents.

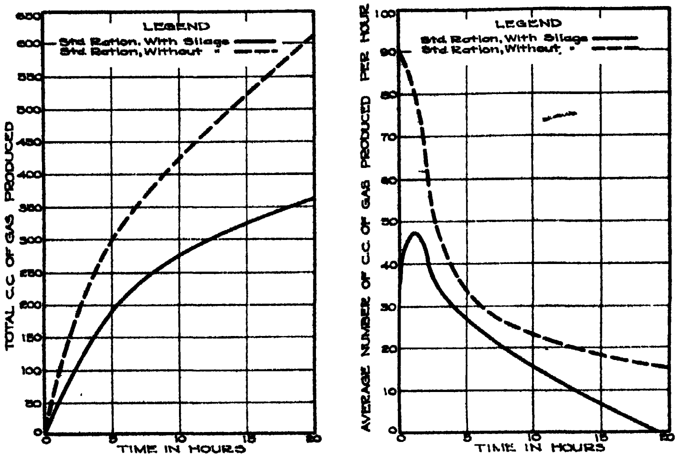

FIG. 1. Gas production from 200 gms. rumen ingesta originating from a standard ration with silage and a standard ration without silage. (Based on readings taken at 1, 2, 3, 4, 5 and 20 hrs. after start of fermentation.)

TABLE 1

Total gas produced by 200-gram samples of ingesta when the cow was fed two typical farm rations

	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	20 hrs.
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
	Typical ration, with silage					
Average of 24 samples	43.7	89.0	126.8	159.1	188.3	361.4
	Typical ration, without silage					
Average of 30 samples	87.2	162.2	219.6	262.7	297.9	609.2

Since *in vitro* trials always showed a steady drop in rate of fermentation, it seemed wise to determine how much of this drop was due to a decrease in available nutrients and how much was due to the accumulation of toxic metabolic products. The effect of inanition is shown in table 2. When feed was withheld from the cow for 24 hours, three-fourths of the normal amount of ingesta in the rumen had disappeared. The remaining ingesta was considerably more alkaline than that of rumen contents where fasting did not occur. The cow from which the ingesta were obtained had access to water at all times. The water ingested and saliva secreted should have removed the major portion of the metabolic products produced by the organisms living in the first two stomachs. Just what percentage of the viable organisms was carried on into the true stomach is not known.

TABLE 2

Effect of withholding feed on rate of fermentation of rumen ingesta as measured by total gas production and hydrogen-ion concentration*

Trial number	Gas production						H-ion concentration	
	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	20 hrs.	0 hrs.	20 hrs.
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*pH*	*pH*
Check trials								
1	70.3	128.0	177.5	216.0	252.3	542.5	6.20	5.25
2	67.0	131.0	185.5	227.5	263.2	566.3	6.59	5.51
3	72.3	134.3	186.8	221.8	252.8	525.7	‡	‡
Ave.	69.9	131.1	183.3	221.8	256.1	544.8		..
Feed withheld for 24 hours								
1	†	†	20.0	25.0	30.0	173.2	7.86	7.25
2	†	†	14.0	22.5	28.3	114.5	8.02	6.92
3	†	18.2	38.3	60.5	70.7	344.5	‡	‡
Ave.	†	18.2	24.1	36.0	43.0	210.7	..	....

* Each figure is an average of six samples.

† Gas volumes so small that readings were highly inaccurate.

‡ No pH readings taken.

These results were compared with dilution of the rumen contents *in vitro* (table 3). Where the amount of ingesta used remained constant little effect was noted on total gas production by adding moderate amounts of distilled or tap water. Although it might be suspected that the additional water would tend to submerge the ingesta in the flasks, actually this did not occur. As soon as gas began to form, the ingesta would quickly rise to the top of the liquid or be "blown" into a less dense mass which extended well above the water line. No attempt was made to agitate the flasks of ingesta regularly although occasionally all flasks were shaken to prevent the material from overflowing the flasks. Preliminary trials indicated that covering the ingesta in the flasks with CO_2 or bubbling air through it only slightly modified fermentation. In all cases fermentation was somewhat increased in the presence of air.

TABLE 3

*Effect of dilution with distilled water on rate of fermentation of rumen ingesta as measured by total gas production**

Trial number	Water added per 200 grams of ingesta	Gas production					
		1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	20 hrs.
		cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
		Check samples					
1	...	54.3	123.3	172.7	223.7	258.0	564.7
2	...	36.3	80.0	126.0	159.0	190.3	504.0
3	...	69.7	124.0	160.3	199.7	237.7	528.7
4	...	70.7	145.7	197.0	238.7	268.7	598.0
5	...	67.7	136.3	196.0	242.0	276.0	597.0
Ave.	...	59.7	121.9	170.4	212.6	246.1	558.5
		Diluted samples					
1	50	50.3	112.3	160.7	208.7	244.3	530.3
2	50	29.5	69.5	114.5	147.5	178.0	499.0
3	50	65.3	117.3	155.0	195.3	230.7	532.3
4	100	42.7	94.3	141.0	176.3	203.0	514.7
5	100	55.0	118.0	173.0	218.0	252.0	594.0

* Each figure is an average of triplicate samples.

When the cow drinks large amounts of water the rumen contents are not only diluted but, in winter at least, the bacteria may be temporarily cooled below the optimum temperature for growth. Probably a more important factor would be changes in temperature of the ingesta due to prolonged

TABLE 4

Effect of temperature on rate of fermentation of rumen ingesta as measured by total gas production and hydrogen ion concentration*

Trial number	Gas production						H ion concentration	
	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	20 hrs.	0 hrs.	20 hrs.
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*pH*	*pH*
	Samples held at 38.5° C. (check)							
1	111.0	191.0	248.3	294.3	333.7	619.0	5.89	5.37
2	108.7	177.0	229.7	272.7	310.7	595.3	5.94	5.44
3	70.0	127.3	174.3	209.3	235.7	483.3	5.76	5.45
Ave.	96.6	165.1	217.4	258.8	293.4	565.9		
	Samples held at 42.5° C.							
1	133.0	215.0	276.0	321.0	357.0	612.7	5.89	5.35
2	140.3	222.3	291.7	344.0	385.7	668.7	5.94	5.45
3	84.0	148.0	196.0	231.0	258.3	472.0	5.76	5.47
Ave.	119.1	195.1	254.6	298.7	333.7	584.5		

* Each figure is an average of triplicate samples.

TABLE 5

Effect of temperature on rate of fermentation of rumen ingesta as measured by total gas production and hydrogen-ion concentration*

Trial number	Gas production						H-ion concentration	
	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	20 hrs.	0 hrs.	20 hrs.
			cc.	cc.	cc.		pH	pH
			Samples held at 38.5° C.					
1	81.3	139.0	179.3	217.7	249.0	506.7	5.90	5.30
2	85.0	154.3	208.3	250.0	285.7	580.0	5.83	5.30
3	76.7	141.3	189.7	226.3	255.3	527.3	5.89	5.48
Ave.	81.0	144.9	192.4	231.3	263.3	538.0		
			Samples held at 40.5° C.					
1	89.3	147.7	188.7	225.0	254.0	473.7	5.90	5.48
2	104.0	180.3	230.0	271.7	305.7	583.7	5.83	5.28
3	89.3	154.0	202.3	235.3	265.3	508.3	5.89	5.29
Ave.	94.2	160.7	207.0	244.0	275.0	521.9		

* Each figure is an average of triplicate samples.

differences in external environmental temperature or to differences in heat production as the result of altered fermentation. By using two constant temperature baths it was possible to study these changes on aliquot samples of ingesta (tables 4, 5 and 6). Changes of two to four degrees in temperature only slightly altered the rate of gas production. Greater changes than

TABLE 6

Effect of temperature on rate of fermentation of rumen ingesta as measured by total gas production and hydrogen-ion concentration*

Trial number	Gas production						H-ion concentration	
	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	20 hrs.	0 hrs.	20 hrs.
			cc.	cc.	cc.		pH	pH
			Samples held at 38.5° C.					
1	60.3	106.7	146.3	176.7	201.7	449.7	5.87	5.51
2	72.3	127.7	170.0	205.0	236.7	466.7	5.98	5.58
3	64.7	121.0	169.0	209.3	243.3	499.0	5.92	5.57
Ave.	65.8	118.5	161.8	197.0	227.2	471.8		
			Samples held at 36.5° C.					
1	45.7	89.0	126.0	152.7	176.7	422.3	5.87	5.50
2	60.7	113.0	149.3	181.0	209.3	438.3	5.98	5.59
3	52.3	100.3	139.3	173.3	205.0	445.3	5.92	5.61
Ave.	52.9	100.8	138.2	169.0	197.0	435.3		

* Each figure is an average of triplicate samples.

this, such as are caused by the drinking of large amounts of cold water, are so temporary that they have not been considered in this study.

As already mentioned, the hydrogen-ion concentration of the rumen contents varies within comparatively wide limits. With the exception of the fasting condition, the pH of the rumen contents studied varied between 5.75 and 6.99 (tables 2, 4, 5, 6, 7, 8, 9 and 11). At the end of 20 hours' fermentation *in vitro* the pH had dropped to 5.5–5.25. Under certain experimental conditions (table 11) the pH rose to 8.42 after 20 hours of fermentation *in vitro.*

Most of the readings taken while the cow was receiving a ration of alfalfa hay and grain were around pH 6.3 whereas with those taken while the cow was receiving bluegrass pasture and grain, only one reading was higher than pH 6.0. These observations would indicate a definitely more acid condition of the rumen ingesta when the cow was on pasture. Furthermore, this decline in pH was accompanied by a 16 per cent reduction in gas production during 20 hours' fermentation of the ingesta *in vitro.*

TABLE 7

Effect of 10 per cent sodium hydroxide on rate of fermentation of rumen ingesta as measured by total gas production and hydrogen-ion concentration*

Trial number	10% sodium hydroxide per 200 grams of ingesta	Gas production						H-ion concentration	
		1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	20 hrs.	0 hrs.	20 hrs.
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*pH*	*pH*
Samples without sodium hydroxide									
1	......	89.0	157.5	208.0	244.0	280.5	526.5	5.80	5.38
2	......	76.0	141.0	186.5	225.0	256.0	527.0	6.03	5.60
3	......	70.0	132.5	176.0	212.0	245.5	516.0	5.86	5.47
Ave.	......	78.3	143.7	190.2	227.0	260.7	523.2	......	. .
Samples with sodium hydroxide added									
1	1	89.5	161.0	219.5	258.0	299.0	579.5	5.80‡	5.50
1†	3	85.5	150.0	200.0	237.5	277.0	642.5	5.80	5.58
2	3	76.0	139.5	188.0	228.5	266.0	647.5	6.03	5.76
2†	10	52.0	95.5	123.5	148.0	171.5	477.5	6.03	6.85
3	20	22.0	31.5	45.5	50.5	55.0	129.5	5.86	6.80

* Each figure is an average for duplicate samples.

† Two experimental series containing different amounts of sodium hydroxide were run concurrently with each check series.

‡ Before addition of sodium hydroxide.

A number of attempts were made to influence the hydrogen-ion concentration *in vitro.* As already mentioned acetic and lactic acid materially decreased gas production. No pH determinations were made in these trials. The adding of agricultural limestone to the samples of ingesta had very little effect on the pH although it did accelerate fermentation (table 8). Small amounts of sodium hydroxide, if anything, slightly increased fermentation (table 7). On the other hand, ten and twenty cubic centimeters of 10 per cent sodium hydroxide raised the pH of the ingesta and definitely inhibited fermentation. Adding as much as 20 cubic centimeters of tenth normal hydrochloric acid to the flasks of ingesta had almost no effect on the rate of gas production (table 9). The pH of the experimental and the check samples were quite similar after twenty hours' fermentation.

TABLE 8

Effect of agricultural limestone on rate of fermentation of rumen ingesta as measured by total gas production[*] and hydrogen-ion concentration

Trial number	Limestone per 200 grams of ingesta	Gas production						H-ion concentration	
		1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	20 hrs.	0 hrs.	20 hrs.
	gms.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*pH*	*pH*
		Samples without limestone							
1		82.0	166.7	224.0	269.3	310.7	694.0		
2		85.7	162.7	213.7	259.0	297.3	538.7		
3		83.7	163.7	230.7	273.0	313.3	722.3	6.48	5.28
4		67.7	134.7	192.7	239.3	276.0	623.0	5.89[†]	5.37[†]
5		65.0	136.7	192.0	230.0	264.0	597.3	5.89[†]	5.48[†]
Ave.		76.8	152.9	210.6	254.1	292.3	635.1		
		Samples with limestone added							
1	0.5	80.0	167.3	225.3	278.0	319.7	744.7		
2	1.0	96.0	180.7	239.3	289.7	332.7	745.0		
3	1.0	107.7	199.3	274.3	323.3	374.7	852.3	6.48[‡]	5.45
4	5.0	80.0	159.3	230.0	290.7	337.7	866.0	5.89[†]	5.46[†]
5	10.0	76.7	163.3	231.0	277.7	327.0	778.0	5.89[†]	5.46[†]

[*] Each figure is an average for triplicate samples.

[†] These readings were not obtained on the same samples that were used for the determination of gas production. However, the treatment was the same.

[‡] Before the addition of agricultural limestone.

In that most simple forms of life depend upon rather fixed osmotic pressures of the milieu in which they live, it seemed reasonable to suspect that changes in the sodium chloride concentration might be important in this study. Besides, a frequently heard suggestion on how to prevent bloat is to add salt to the drinking water. However, results obtained in our experiments would tend to discourage this practice (table 10). Amounts of salt which the cow might tolerate in the drinking water, as determined by tasting, had no inhibitory effect on gas production by the rumen flora. In fact, the tendency was toward an acceleration in gas production. Other home remedies, such as equal parts of soda, salt and limestone, had no inhibitory effect on these organisms as measured by gas production. Limestone noticeably stimulated fermentation.

TABLE 9

Effect of N/10 hydrochloric acid on rate of fermentation of rumen ingesta as measured by total gas production and hydrogen-ion concentration*

Trial number	N/10 HCl per 200 grams of ingesta	Gas production						H-ion concentration	
		1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	20 hrs.	0 hrs.	20 hrs.
		cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*pH*	*pH*
Samples without hydrochloric acid									
1	...	168.0	265.0	327.5	383.0	433.5	736.0	6.64	5.49
2	......	104.0	167.5	218.0	264.0	303.5	637.0	5.90	5.39
3	......	102.0	175.7	239.9	290.7	331.3	632.3	6.44	5.24
4	......	104.7	205.3	266.0	312.0	347.7	700.0	6.64	5.40
5	......	121.0	205.7	269.3	316.7	358.0	725.7	6.34	5.32
Ave.	......	119.9	203.8	264.1	313.3	354.8	686.2	..	..
Samples with hydrochloric acid added									
1	0.3	174.7	275.0	340.0	400.0	450.0	779.0	6.64†	5.60
2	1.0	106.3	172.3	224.3	269.7	310.7	657.3	5.90	5.38
3	3.0	95.3	169.0	229.3	276.0	314.7	618.7	6.44	5.21
4	10.0	110.0	213.7	275.7	321.0	362.0	700.0	6.64	5.29
5	20.0	110.7	192.7	250.3	296.3	337.3	677.0	6.34	5.17

* Each figure in the check samples of trials 1 and 2 is the average of duplicate samples. All others are averages for triplicate samples.

† Before addition of HCl.

TABLE 10

*Effect of sodium chloride on rate of fermentation of rumen ingesta as measured by total gas production**

Trial number	Sodium chloride per 200 grams of ingesta	Gas production					
		1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	20 hrs.
	gms.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
Samples without sodium chloride							
1	....	109.3	177.0	248.0	286.7	321.7	662.3
2	......	83.0	162.3	210.7	251.3	286.0	611.3
3	......	105.7	188.0	248.3	293.3	333.3	603.3
4	......	87.0	160.7	209.7	246.7	282.3	610.7
5	......	88.0	158.0	211.7	256.7	287.7	623.3
Ave.	.....	94.6	169.2	225.7	266.9	302.2	622.2
Samples with sodium chloride added							
1	0.5	123.3	206.0	268.3	307.7	349.3	692.7
2	1.0	82.3	159.7	204.7	248.7	279.3	595.7
3	1.0	110.0	194.0	257.0	302.3	341.7	623.7
4	5.0	77.0	141.3	186.3	218.0	250.7	483.3
5	10.0	78.3	138.0	186.0	226.7	251.7	497.7

* Each figure is an average of triplicate samples.

Quite a number of preliminary trials were run with other materials in the hope of throwing more light on the physiology of excessive gas production in the rumen. Molasses and a few pure sugars were tried. These supposedly good bacterial foods tended to inhibit gas production temporarily. Longer trends were not studied *in vitro* although it was noticed that the rumen contents became more frothy when molasses was fed regularly to the cow. Vitamins such as thiamin chloride, ascorbic acid and calcium pantothenate when used singly in preliminary trials showed no marked effect on gas production. Glycerol and urea only slightly modified fermentation when small amounts were used. However, ten grams of urea per flask completely inhibited gas production.

One of the more interesting compounds tried was sodium formate (table 11). This material, in all amounts used, very definitely stimulated fermentation and raised the pH of the fermented material. Calcium formate, sodium lactate, calcium lactate and calcium gluconate gave similar but less marked increases. More detailed study will be necessary to clarify the results. Although it is probably as necessary to provide a "balanced ration" for bacteria as for dairy cows, very few of these compounds were tried in combination, principally due to the lack of time. Milk was tried and in limited amounts tended to increase fermentation about 30 per cent. The authors were not especially interested in drugs which would inhibit fermen-

TABLE 11

Effect of sodium formate on rate of fermentation of rumen ingesta as measured by total gas production and hydrogen-ion concentration*

Trial number	Sodium formate per 200 grams of ingesta	Gas production						H-ion concentration	
		1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	20 hrs.	0 hrs.	20 hrs.
	gms.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*pH*	*pH*
Samples without sodium formate									
1		122.3	199.0	251.7	292.3	330.0	742.0		
2		98.3	184.3	243.7	291.7	327.7	663.7	6.06†	5.43†
3		100.7	191.7	244.3	296.0	332.3	683.3	5.75†	5.29†
4		92.0	169.7	224.3	276.0	308.0	558.7	5.78†	5.48†
5		129.7	205.3	260.3	305.0	346.0	705.3	6.65	5.48
Ave.		108.6	190.0	244.9	292.2	328.8	670.6		
Samples with sodium formate added									
1	0.5	151.7	246.0	314.7	380.3	430.3	965.3		
2	0.5	118.0	228.0	301.7	368.3	421.0	876.7	6.06†	7.00†
3	1.0	130.3	251.3	326.0	399.3	459.7	1042.3	5.75†	7.47†
4	1.0	120.0	217.3	290.7	357.3	415.3	840.3	5.78†	7.17†
5	2.0	166.7	274.0	361.7	428.7	485.0	1158.0	6.65	8.42

* Each figure is an average of triplicate samples.

† These readings were not obtained on the same samples that were used for the determination of gas production. However, the treatment was the same.

tation as it was felt that fermentation is a normal physiological process which must be encouraged if the cow is to derive the maximum good from her feed, especially high fiber feeds.

One of the chief reasons for starting this investigation was to learn why cows bloat more readily on legume pasture than on any other feed and why some cows bloat on legume pasture under one set of conditions and not under another. Probably the most surprising thing we found was that similar amounts of bluegrass and green alfalfa when partially submerged in aliquot samples of rumen fluid produced almost the same amount of gas. In fact the rates of gas production tended to follow the dry matter content of the materials used even when the grass was dried before immersing in the rumen fluid. Unfortunately, varying amounts of material were used in this set of trials and the time at which readings were made did not coincide in each trial so the data cannot be readily summarized in table form. Freshly frosted alfalfa and alfalfa covered with dew when cut was also tried but no acceleration in rate of fermentation was noted. Even freezing green alfalfa and then grinding it while frozen did not alter the end results beyond the limits of experimental error. Besides the *in vitro* trials, an effort was made to change the cow to feed which had been frosted but this did not prove practical. However, it was found that the dry matter content of the rumen ingesta was not materially different when the cow was on dry or green feed.

No determinations were made of the gas as produced. Analyses of the total gas produced in 20 hours showed a carbon dioxide content of around 50 per cent.

An attempt was made to induce bloat by turning cows into a field of young alfalfa. These cows refused to eat alfalfa for any extended time with the result that conditions favorable for bloat could not be obtained. It was noted, however, that at first these cows were eating the fresh alfalfa at the rate of one pound per minute while the best rate at which they ate good bluegrass pasture was one-third of a pound per minute. It might be added in explanation of this difference in rate of ingestion that fresh alfalfa is more or less pinched off by the cow in grazing while bluegrass must be gripped firmly and the grass cut off against the sharp edges of the lower teeth. The data on rate of grazing were obtained by weighing the cow immediately before and after grazing. All excreta voided was collected and corrected for.

DISCUSSION OF RESULTS

From the results obtained it is evident that temporary changes, though of a substantial nature, can be made in the environment of bacteria in the rumen without seriously affecting the rate of gas production. Long-time trends were not of interest in this experiment because of unpredictable changes in bacterial flora which might also occur.

Nothing in these results would indicate that the rate of fermentation in the rumen can be increased precipitously by the addition of many of the more common sugars, minerals, or vitamins associated with proper nutrition. Even under normal conditions gas is generated too rapidly to be absorbed without a frequent eructation of gas. In fact, our results would indicate that green alfalfa does not stimulate gas production any more than green growing bluegrass. This has led us to seek a physical explanation for bloat. Because our cows ate alfalfa three times as fast as bluegrass, added weight is given to this explanation.

The fact that injury from fermentation in the paunch never occurs with cows on legume pasture unless they bloat, would tend to indicate that the gases formed are not especially toxic. Most cows will be seen to eruct gas two or more times per minute while grazing. True, under increased pressure the rate of absorption of the gases produced is much greater and may be a contributing factor to death from bloat. However, the failure of the gas to escape normally appears to be the predisposing factor.

Roughage as ingested is dropped from the cardia into the recticulum and anterior compartment of the rumen. By running bluegrass and alfalfa through a meat chopper to simulate chewing, it was found that chopped alfalfa forms a much more compact mass than chopped bluegrass. At the rate of grazing actually observed with cows which had been without food for 12–18 hours it is quite possible that such animals might ingest 50–100 or more pounds of this succulent feed before resting. This amount of material if remaining in a relatively compact mass would tend to force the stomach down against the abdominal floor. And since the only entrance or exit to the stomach is at the esophageal groove anything which would tend to depress the esophageal groove below the level of the fluid in the fore stomachs would tend to cause trapping of the gas within the rumen and bloating. This condition would be aggravated by the cow lying down after grazing to rest and chew her cud. As fermentation began to increase the freshly eaten mass would be buoyed up by the generated gas and might help to block the escape of the gas. Increased pressure would only seal the exit more firmly. The drinking of water might tend to help wash part of this freshly eaten mass back into the rumen where it would normally be carried by the peristaltic contractions of the reticulum. On the other hand, if the mass was not dislodged the water would only make it more difficult for the gas to escape.

By palpation of the fore stomachs through a rumen fistula one finds that the reticulum contracts periodically with considerable force. This peristaltic wave throws the fluid in the reticulum over the more solid material in the rumen. A slow peristaltic wave then passes over the rumen and tends to squeeze out much of this fluid, taking with it some of the soluble material freed by soaking, maceration and bacterial action. The semi-liquid material flows forward into the reticulum. When a cow quickly ingests large amounts of feed which compacts readily there appears to be danger of upsetting this normal physiological process. Certainly the danger of bloating is greatest immediately after grazing.

This theory would account for the greater prevalence of bloating when cattle graze alfalfa covered with dew or frost by assuming that moistened feeds can be ingested more rapidly than unmoistened feeds. Less saliva would also be added to the fermenting material. As has already been mentioned, it was impossible to get any of the cows, including the one with the rumen fistula, to eat sufficient alfalfa during the experiment to simulate the conditions described.

SUMMARY

Ingesta obtained from a cow by means of a rumen fistula were fermented *in vitro* and the gas collected over twenty-hour periods. Ingesta obtained after the cow had eaten silage produced less gas per gram of dry matter than ingesta obtained when the cow received only hay and a mixed grain ration. Bluegrass pasture and grain produced an ingesta definitely more acid than did alfalfa hay and grain. The ingesta normally became more acid as fermentation progressed. This increase in acidity was accompanied by a decline in rate of gas formation.

When feed was withheld from the cow for 24 hours, her rumen was not more than one-fourth full and most of the material present was in a semiliquid state. Ingesta obtained at this time was alkaline and the rate of gas production was very slow.

Aliquot samples of the rumen contents were treated in different ways and the rate of fermentation compared. Changes in dilution, temperature and hydrogen-ion concentration, in the ranges normally occurring in the stomach, had little effect on the rate of fermentation. Amounts of salt which the cow would tolerate in her drinking water also had little effect in *in vitro* studies. Common minerals, such as limestone, accelerated fermentation probably through neutralization of the acids formed. Of a number of other materials tried, acetic and lactic acids tended to depress fermentation most severely while sodium formate had the greatest accelerating influence.

Finding no basis for bloat as a result of altering the environment of the bacteria in rumen ingesta, the authors propose a theory based upon the physical effect of the feed. This theory was not verified experimentally due to a lack of cooperation by the cows under observation.

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PREVENTION OF DEVELOPMENT OF HYDROLYTIC RANCIDITY IN MILK

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Hydrolytic rancidity in milk manifests itself by imparting the somewhat bitter taste and the sharp, unpleasant aroma characteristic of fat acids of low molecular weight, especially butyric acid. A partial hydrolysis of milk fat, which is responsible for rancid flavor, can be brought about by two distinct sets of conditions.

1. Hydrolysis will result from certain specific treatments of raw milk such as (a) homogenization (1), (b) violent shaking of warm milk (5), (c) warming of the precooled milk to about 30° C. and cooling again below 10° C. (4). These treatments of milk result in the activation of lipase, an enzyme seemingly present in all raw supplies of milk (3). Whether one or more lipolytic enzymes are involved in the hydrolysis of milk fat by activation treatments is a question (2); but normally, in any case, the rancid flavor does not appear unless the milk is subjected to one of the treatments mentioned above. As was pointed out by Tarassuk and Richardson (10), lipase-activation treatments of milk have one property in common: they lead to disruption, partial replacement, or distortion of the natural adsorption layer on the fat globules; and therein lies the clue to the mechanism of activation.

2. During winter feeding when cows have no access to green feed, the milk from some cows (usually those that are late in lactation) contains a naturally active milk lipase. This milk exhibits a spontaneous lipolysis of fat since no involved treatment is necessary in order to make it become rancid upon aging. The only condition necessary for the activation of this lipase is cooling (9). Once the milk has been cooled, lipase activity is not materially affected whether it is aged in the cold or rewarmed immediately to 20°, 30° or 37° C. and aged at those temperatures. The critical cooling temperature is between 20° C. and 15° C., and the rate of lipase action is increased with progressive cooling to lower temperatures.

The extent of occurrence of spontaneous rancidity in milk is reportedly from 3.2 (6) to 35 per cent (7) of milk sampled at the delivery plant as being rancid. The present available methods for preventing the rancidity caused by naturally active milk lipase are (a) pasteurization shortly after milking, and (b) detection of cows whose milk will become rancid on cooling and aging, and the elimination of their milk from the marketable supply. The first method of prevention is usually impracticable or impossible; and the

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second is uneconomical, especially now that a national effort is being put forth to increase milk production.

In the present study a new method of preventing spontaneous lipolysis is presented. This method obviates the economic loss and is simple enough to be practicable.

EXPERIMENTAL RESULTS

The lipolysis of milk fat caused by a naturally active lipase in milk takes place upon cooling and aging. When the concentration of lipase is sufficiently high, the rancid flavor becomes perceptible on aging for only 3 hours. In such milk the rate of lipolysis, according to Tarassuk (8), is highest in the first 10 hours. The further rate of hydrolysis by lipase is progressively reduced by accumulation of the products of hydrolysis and by lowering of the pH. As is well known, if such milk after cooling and aging for 24–36 hours is mixed with a normal milk, the admixture of even 3–5 per cent of it will impart a rancid flavor to the entire mixture.

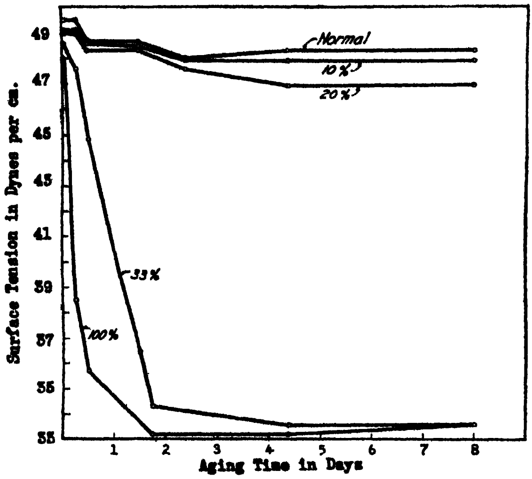

FIG. 1. Rate of lipolysis in the mixtures of normal milk and milk containing naturally active lipase in very high concentration.

In this work the progress of lipolysis was studied in the various mixtures of normal milk and milk containing naturally active lipase when the two are mixed within less than one hour after milking—that is, before the development of rancidity in potentially rancid milk.

The rate of lipase action was determined by measuring the surface tension of milk (8) with a Du-Noüy tensiometer at 20° C. The mixing was done within an hour after milking, before cooling or immediately after cooling. All samples were brought to 3–5° in ice water and aged at 5° C. The surface tension was measured at intervals during aging. The surface-tension data were supplemented also by organoleptic test of milk by two experienced judges.

Figures 1, 2, and 3 illustrate the data on the rate of hydrolysis of milk fat by a naturally active lipase when the various milks containing this lipase on different occasions and from different cows were mixed with a normal milk[1] within an hour after milking. The percentage as shown on the curves refers to the per cent of potentially rancid milk that was added.

Figure 1 illustrates the rate of hydrolysis of milk fat in milk containing a very high concentration of a naturally active lipase. As can be seen from the curve (100 per cent) in figure 1, the hydrolysis is nearly completed within twelve hours. This particular milk tested rancid within two hours after cooling; yet when 20 per cent of this milk was mixed with a normal milk within one hour after milking, the mixture failed to develop the rancidity perceptible by organoleptic test on aging for nine days. Judging from the data in this figure, there is a critical concentration of lipase below which the rate of hydrolysis falls to such extent that the presence of lipase in concentrations below the critical one has no practical importance. The hypothesis of critical concentration of lipase is supported also by figures 2 and 3. In figure 2 the lipolytic activity of "lipase milk" is also unusually high, although less than that shown in figure 1. In this case an admixture of 75 per cent of normal milk delayed the development of perceptible rancid flavor for three days—an average life of market milk. The mixture of 20 per cent of "lipase milk" and 80 per cent of normal milk failed to develop a rancid flavor on aging for as long as fifteen days.

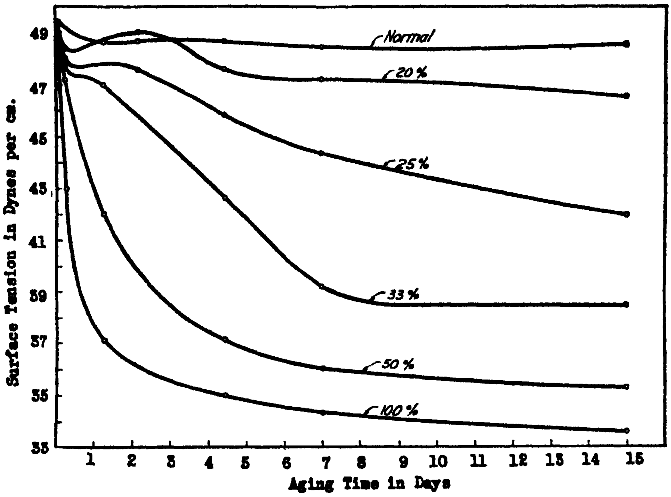

FIG. 2. Rate of lipolysis in the mixtures of normal milk and milk containing naturally active lipase in high concentration.

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[1] The term "normal milk" as used here means milk that will not show any appreciable hydrolytic rancidity on cooling and aging for three days, as can be determined by organoleptic test or by surface-tension measurements.

Figure 3 represents the average lipolytic activity of milk that goes rancid spontaneously. As can be seen from the rate of hydrolysis, such milk shows a pronounced rancidity on cooling and aging for 24 hours or longer. As is evident from figure 3, the development of rancid flavor in such milk can be prevented for a period longer than the average life of milk by mixing it with a normal milk in a proportion even as great as 1 : 1. The average organoleptic score given by two judges to the mixtures of the milks represented in figure 3 appears in table 1. The samples were keyed and scored as unknowns placed in random order.

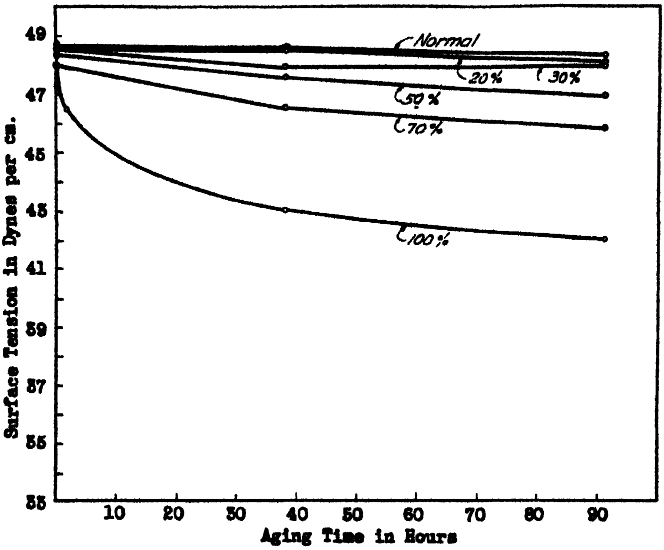

FIG. 3. Rate of lipolysis in the mixtures of normal milk and milk containing naturally active lipase in average concentration.

TABLE 1

*Organoleptic score of mixtures of normal and potentially rancid milk after various aging periods**

Sample	Time of Aging		Criticism
"Lipase milk" in milk	38 hours	91 hours	
per cent			
100	0	0	Very rancid
70	18	16	Slightly rancid and rancid
50	23	23	No criticism
30	23	23	No criticism
20	23	23	No criticism
0 (normal)	23	23	No criticism

* The milk-score card of the American Dairy Science Association allows 25 points for a perfect flavor score. By custom, milks with no flavor defects are given a score of 23 to 24.

The data demonstrate clearly that the rate of lipolysis of milk fat due to a naturally active lipase does not follow the rate of dilution of the enzyme when a dilution is made by mixing with normal milk. In general the lipolysis curves at higher concentration of the enzyme have the characteristics of an exponential curve. When the original "lipase milk" is diluted 1:3 or higher with a normal milk, the rate of lipolysis after the first few hours of aging approaches a straight line, which is rather characteristic of a normal milk.

Since very seldom will more than one out of five cows in a herd give milk that goes rancid spontaneously, the mixing of the herd's milk will prevent the development of rancid flavor. This method of preventing hydrolytic rancidity in milk is not equally applicable to a separated cream. The experimental work is being done to study this phase of the problem.

This method of mixing also cannot be applied for the prevention of the development of rancidity resulting from activation of lipase of normal milk by temperature changes as stated previously. In the latter case the rate of lipolysis of milk fat seemingly follows the rate of dilutions and the details of this study will be reported in a subsequent paper.

SUMMARY

The development of hydrolytic rancidity in raw milk by a naturally active lipase present in high concentration can be successfully prevented by mixing with normal milk within an hour after milking. The amounts to be mixed depend upon the concentration of the lipase. The mixing of "lipase milk" with normal milk in a proportion of 1:4 or higher will always prevent rancidity. To insure the effectiveness of this method the mixing must be made within an hour after milking, before cooling or immediately after cooling. If a milk containing a naturally active lipase is allowed after cooling to age separately and thus to become rancid, then the addition of very small amount to a normal supply will impart the rancid flavor to the whole mixture.

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SHARK LIVER OIL AND THE VITAMIN A POTENCY OF MILK*

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INTRODUCTION

The vitamin A activity of winter milk usually is lower than that of summer milk due to the reduced carotene intake of cows maintained on winter rations. It is well known that the vitamin A activity of milk is dependent on the level of carotene and vitamin A in the ration. The data presented herein are the result of an attempt to increase the vitamin A potency of winter milk.

REVIEW OF LITERATURE

Reports in the literature have shown that feeding carotene, whether as green pasture or hay in amounts as high as 6,000,000 International Units (I.U.) of vitamin A per cow per day, has never resulted in butter containing more than 56 I.U. of vitamin A per gram, nor milk containing over 2500 units per quart (2, 18, 20, 21, 22).

Archibald and Parsons (1) fed a fortified cod-liver oil with the dairy ration and reported a significant increase in the vitamin A level of the milk from these animals as compared with those receiving unsupplemented rations. They reported 3596 units of vitamin A per quart of milk when cod-liver oil (3000 U.S.P. units of vitamin A per gram) was fed at 0.25 per cent level in the grain. The vitamin A assays of the milk in two consecutive years did not agree because of a change in the quality of the roughage used in the basal ration. These investigators noticed a favorable effect on milk production and found no significant effect on the average butterfat content of the milk.

Deuel *et al.* (3, 4) administered 700,000 and 1,400,000 I.U. of vitamin A in the form of shark-liver oil to Guernsey cows and observed a corresponding increase in the vitamin A potency of the milk, proportional to the amount of vitamin fed. The basal diet included large amounts of fresh-cut alfalfa and baled alfalfa hay. These workers reported an average of 113 I.U. of vitamin A per gram of butterfat after daily administration of 1,400,000 I.U. of vitamin A as shark-liver oil, and as high as 172 I.U. of vitamin A per gram of butterfat with one animal. These analyses were

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The data in this paper were taken in part from a Doctor's thesis submitted by H. E. Skipper to the Graduate School of the University of Florida, June 1941.

colorimetric, using a Bills-Wallenmeyer electronic photometer, although bioassays on a few samples showed a slightly lower potency. Under the conditions of their experiment apparently no threshold level was reached. They reported also a 10 per cent increase in the milk production of the cows receiving shark-liver oil and a slightly greater increase of butterfat. In a second experiment these workers reported a potency of 55 I.U. of vitamin A per gram of butter from cows receiving shark-liver oil.

It has been reported that high concentrations of cod-liver oil decreased the percentage of butterfat (5, 7, 11, 12, 13); that cod-liver oil concentrate had no effect (1); that shark-liver oil either had no effect (11), increased (4), or decreased the percentage of butterfat (17). Guthrie (6) reported that a high vitamin A ration would increase the vitamin C content of the milk. The effect of feeding shark-liver oil in the ration on the vitamin A potency, butterfat percentage, and ascorbic acid content of milk will be reported herein.

EXPERIMENTAL METHODS

Ten Jersey cows selected from the Florida Agricultural Experiment Station dairy herd were used. They were selected as nearly uniform as possible as regards milk and butterfat production, and stage of lactation. These cows were placed in four groups: Groups I, II, and III, consisting of two animals each, and a control group containing four animals. The experiment was divided into three feeding periods: a preperiod before supplementation with shark-liver oil, a dry lot or winter period with oil supplementation, and a pasture or spring period with oil supplementation.

The basal ration was the regular dairy herd ration which consists of a well-balanced concentrate mixture and silage made from corn, well matured and well eared. During the winter period, Alyce clover (*Alysicarpus vaginalis* (L.) DC.) hay was added to the basal ration at the level of approximately eight pounds per head daily. The control group received only the basal ration, whereas Groups I, II and III received 2.5, 5.0 and 10 pounds of shark-liver oil (9,000 I.U. of vitamin A per gram), respectively, per ton of the mixed concentrates. The shark-liver oil was incorporated in the wheat bran which was then mixed with the other constituents of the concentrate mixture. Fresh batches of feed were prepared at 30-day intervals. The cows received approximately 10 pounds of the concentrate mixture and 20 pounds of corn silage per head daily.

The shark-liver oil and Alyce clover hay were assayed biologically for vitamin A by the U.S.P. XI technique (15). The carotene contents of the basal ration, corn silage and Alyce clover hay were determined by the method of Peterson, Hughes and Freeman (14).

Composite milk samples for a 24-hour period were collected from each cow at the end of each experimental period. The ascorbic acid content of each milk sample was determined immediately after collection by the method of

Sharpe (19). The butterfat content of each milk sample was determined by the Babcock method. The milk from cows of the same group was then mixed, heated to 143° F. for ten minutes, homogenized at 43,000 pounds pressure per square inch, placed in half-pint carton containers and stored at 0° F. awaiting bioassay.

In previous biological assays of milk for vitamin A, it was found that 0.5 cc. per rat daily resulted in a large percentage of deaths before the end of the assay period. Therefore, 1 cc. of milk per rat daily was the level fed.

A growth-response curve for the U.S.P. reference cod-liver oil was established for the rat strain used in this laboratory. The growth responses on the milk samples were compared with this reference growth-response curve, as were the growth responses of the rats on shark-liver oil and Alyce clover hay.

RESULTS

The shark-liver oil assayed at least 9,000 I.U. of vitamin A per gram, Alyce clover hay approximately 170 I.U., and the corn silage 1.4 I.U. The basal concentrate mixture showed no appreciable vitamin A activity.

Table 1 gives the approximate daily vitamin A content of the rations for the different groups of cows receiving shark-liver oil, and the vitamin A potency per quart of milk from these groups of animals for the different periods.

TABLE 1

The vitamin A potency per quart of milk from cows receiving shark-liver oil

Group	Supplement of oil per ton of mixed concentrates	I.U.* of vitamin A per day in the ration	Vitamin A potency per quart of milk		
			Pre-period	Dry lot period	Pasture period
	lbs.		*I.U.*	*I.U.*	*I.U.*
Basal†	0.0	744,000	1740	1570	1730
I	2.5	795,000	1880	1845	2035
II	5.0	846,000	1845	1890	1880
III	10.0	948,000	1770	1910	1875
Cow #601	6 oz. per day	2,144,000			1750

* I.U. = International unit of vitamin A.

† Basal ration

	I.U. of Vitamin A per cow per day
Grain mixture	0
Corn silage	127,000
Alyce clover hay	617,000
	744,000

The feeding of high levels of shark-liver oil slightly lowered the percentage butterfat of milk as shown in table 2.

The ascorbic acid content of the milk samples from each cow ranged from 17.2 to 20.4 mg. per liter during the control period; 15.0 to 19.8 mg. per liter during the dry lot period, and 17.7 to 25.3 mg. per liter during the period

TABLE 2

The relation of high intakes of shark-liver oil to the percentage butterfat of milk from Jersey cows

Cow No.	Level of oil fed daily	Percentage of butterfat				
		Before feeding oil	During and after oil feeding			
			Weeks			
			1	2	3	4
	oz.					
601	4	5.1	5.0	5.2	4.7*	5.4
637	6	4.0	4.75	3.8*	4.7	.. ...
539	6	3.7	2.2	3.1*	3.5	. . ..
564	6	4.5	3.7	4.3*	4.7	.. ...
602	6	4.8	4.75	4.7*	5.9	.... .
435	6	5.05	4.2	4.1*	4.65	..

* Feeding of shark-liver oil ceased after taking milk sample.

on pasture. Of the 30 samples a low value of 15.0 mg. of ascorbic acid was found in Lots I and III during the dry lot period, and the high value of 25.3 was found in Lot II in the spring period.

DISCUSSION OF RESULTS

In the preperiod and during the spring period the animals had access to succulent green pasture so that it was impossible to calculate the amount of vitamin A received in the form of carotene.

The most dependable vitamin A potencies of the rations are thus found for the dry lot or winter period, as indicated in table 1. These values ranged from 744,000 I.U. for the animals receiving no supplement of oil, to 948,000 I.U. with oil supplementation. The vitamin A potency of the milk ranged from 1570 to 1910 I.U. per quart. The addition of 0.125 per cent of shark-liver oil in the concentrates of the basal ration (Group I) raised the vitamin A intake from 744,000 to 795,000 I.U. per cow daily. This level of vitamin A in the ration was sufficient to produce milk of maximum vitamin A potency. Increasing the vitamin A potency of the ration above this level by further additions of shark-liver oil (0.25 and 0.50 per cent) did not further increase the vitamin A potency of the milk. Even when these animals were allowed to go on pasture in the spring period, receiving still more vitamin A in the form of carotene, no further increase in the vitamin A potency of the milk was observed. One cow (# 601) received six ounces of shark-liver oil in addition to the basal ration so that the total vitamin A in the ration amounted to 2,144,000 I.U. Even this very high level did not affect the vitamin A potency of the milk beyond that resulting from a ration containing 795,000 I.U. per day. Evidently, a threshold level for vitamin A secretion exists above which more vitamin A in the feed does not increase the vitamin A potency of the milk. The value, 795,000 I.U. of vitamin A per cow per day, closely approaches the value of 550,000 Sherman-Munsell Units, or 770,000 I.U. (1.4 conversion factor) as reported by Wilbur, Hilton and Hauge (21) as necessary in the form of carotene to keep the vitamin A potency of butterfat at a maximum. Deuel *et al.* (3, 4) on the other hand, report no such threshold level in their experiment using Guernsey cows. They found a corresponding increase in the vitamin A potency of the butterfat as the amount of shark-liver oil in the ration increased, reporting as high as 172 I.U. of vitamin A per gram of butterfat (8600 I.U. of vitamin A per quart of milk, on the basis of five per cent butterfat).

It can be noted from table 1 that a significant decrease occurred in the vitamin A potency of the milk from cows of the basal group when pasture was eliminated from the ration during the dry lot period. An increase in the vitamin A potency of the milk for this group during the following pasture period shows the effect of good pasture on the vitamin A activity of milk.

Per cent of butterfat: The administration of four to six ounces of shark-liver oil daily to Jersey cows tended to decrease the percentage of butterfat slightly without affecting the quantity of milk produced. An increase of percentage of butterfat was observed with each animal on cessation of oil administration, as shown in table 2. McCay and Maynard (11) reported that the depressing effect on butterfat secretion caused by feeding cod-liver oil did not occur when shark-liver oil was used, while Deuel *et al.* (4) reported an increase of about 10 per cent in butterfat and milk production with the administration of shark-liver oil. Rupel, Boyer and Phillips (17) have recently reported that large amounts of shark-liver oil decreased the percentage of butterfat at the beginning of the oil feeding period. These data do not confirm the work of Deuel *et al.* (3, 4).

Ascorbic acid: Ascorbic acid is known to be synthesized within the body of the cow. Several investigators (8, 9, 10, 16) have observed that no relationship exists between ascorbic acid in feed and in milk, while Guthrie (6) in 1939 reported that the addition of vitamin A in the form of cod-liver oil increased the ascorbic acid content of milk. There were no indications that the vitamin A activity of the ration influenced the ascorbic acid content of the milk under the conditions of the experiment reported herein.

SUMMARY AND CONCLUSIONS

The vitamin A potency of milk, obtained from cows receiving 744,000 to 2,144,000 International Units of vitamin A per day in rations by the supplementation with shark-liver oil, ranged from 1570 to 2035 I.U. per quart.

The feeding of approximately 795,000 I.U. of vitamin A (0.125 per cent shark-liver oil in the concentrates) per cow daily resulted in milk of maximum vitamin A potency. It is suggested that a threshold level exists for the secretion of vitamin A in milk, since feeding higher levels of vitamin A did not result in further increases of this vitamin in the milk.

Administration of high levels (4 to 6 ounces) of shark-liver oil per cow daily tended to depress slightly the percentage of butterfat in the milk without affecting milk production.

There was no indication that high vitamin A potency of the ration influenced the ascorbic acid content of the milk.

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The authors are indebted to the late Dr. L. M. Thurston and to Irving I. Rusoff for the ascorbic acid determinations of the milk samples; to Security Mills for funds to defray part of the expense of the experiment; and to Shark Industries, Inc., for furnishing the shark-liver oil.

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SEASONAL VARIATION IN SEMEN QUALITY OF THE DAIRY BULL[1]

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It has long been recognized that in many vertebrates spermatogenesis is not continuous but tends to occur at one rather definite period of the year. Cyclic spermatogenic activity is characteristic of the majority of mammals with the exception of such domesticated and semidomesticated species as the guinea pig, rabbit, rat, dog, boar, ram, stallion, bull and man. These species have long been classified as continuous breeders but evidence is now accumulating to show that although spermatozoa are produced throughout the entire year certain seasonal trends in the rate of spermatogenesis and the degree of fertility are evident. The purpose of this study was to determine the influence of season upon semen quality in the dairy bull.

REVIEW OF LITERATURE

The factors influencing spermatogenic activity have been thoroughly reviewed by Moore (10) 'but in brief, the testis is regulated by the gonadotopic hormones of the anterior pituitary gland and to some extent by other endocrines, by the amount and quality of light, by environmental and testicular temperatures and by the plane of body nutrition.

Radulescu (11) in Europe and Roux and Hoffman (12) in South Africa concluded that in the ram spermatogenesis is continuous throughout the year. McKenzie and Berliner (8) in an extensive study of semen production of Hampshire and Shropshire rams under Missouri conditions reported that although spermatozoa were produced during all months, definite seasonal trends in semen quality were apparent. Maximum semen quality was observed in the Shropshires from October to January and in the Hampshires from August to January. Seasonal changes were more apparent in the Shropshire rams and very poor semen was produced by the males of this breed during July and August.

Many studies of spermatogenesis, semen production, sperm physiology and fertility have been made in the bovine, Williams (16), Donham *et al.* (2), Lagerlöf (7), Milovanov (9), Davis and Williams (1), Herman and Swanson (5) and others, but none have reported seasonal differences in semen quality.

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Erb, Wilbur and Hilton (3) in a study of the breeding efficiency of the Purdue Dairy Herd from 1920 to 1940 found that maximum efficiency was obtained in May (74.3 per cent) and that the minimum efficiency occurred in August (58.2 per cent). The causes of the seasonal differences were not apparent from the available records and the present study was undertaken in the hope that information on the rôle of season and its influence on the reproductive performance of the dairy bull might be gathered.

MATERIALS AND METHODS

Four Holstein, two Jersey, two Guernsey and one Ayrshire bulls were used in this study. These animals were regular herd sires in the Purdue University Dairy Herd and ranged in age from one to seven years.

The bulls were housed in individual 10 × 12 foot stalls and had access at will to 12 × 60 foot exercising pens, although no compulsory exercise was employed. The animals had no access to pasture and were maintained throughout the experiment on a grain ration composed of 200 pounds of yellow corn, 100 pounds of oats, 50 pounds of bran, 50 pounds of old process linseed oil meal, 50 pounds of cottonseed meal, 12 pounds of salt and 100 pounds of bone meal. The grain mixture contained 13.4 per cent crude protein and was fed in sufficient amounts to maintain satisfactory growth of the immature bulls and to maintain all of the animals in "good breeding condition" according to the usual Dairy Husbandry standards. First cutting No. 2 grade alfalfa hay averaging 19 per cent crude protein was fed *ad libitum.*

Semen was collected from all bulls with an artificial vagina once each week and, with a few exceptions, two ejaculates were obtained within a ten-minute interval at each collection period.

The methods of semen evaluation were those which are commonly used in investigations of this type and have been fully described by Weisman (15), Lambert and McKenzie (6), McKenzie and Berliner (8) and others. Semen volume and the initial motility of the spermatozoa were immediately recorded and pH determinations were made with the Beckman pH Meter and the one-drop glass electrode within the first post-collection hour. The semen samples were stored at 40° F. in 4 cc. glass vials fitted with paraffined corks and small portions were withdrawn at 24-hour intervals and examined for motility in a stage incubator at 100° F.

The degree of motility of the spermatozoa was expressed in terms of the following criteria:

0 = No motility.

1 = Less than 20 per cent of the sperm showing progressive motion.

2 = Twenty to 40 per cent of the sperm exhibiting progressive motion but no evidence of waves or eddies in the semen drop.

3 = Forty to 60 per cent of the spermatozoa showing vigorous progressive movement and the formation of slowly moving waves in the semen.

4 = Sixty to 80 per cent of the sperm exhibiting rapidly moving waves showing some dead spermatozoa carried along by the motion of the semen.

5 = Eighty to 100 per cent of the spermatozoa showing rapid, progressive movement. The rapidly moving waves formed by the activity of the sperm show very few dead cells being swept along as inert bodies.

The concentration of the spermatozoa per unit volume of semen was determined with the hemacytometer using the technique which is ordinarily applied for the counting of red blood cells. The total spermatozoa per ejaculate were determined and a total motility rating score was obtained by summation of the individual motility ratings during the entire survival period.

Smears of each ejaculate were made at the time of collection, were air dried, and later stained with eosin. These slides were examined at 440 × magnification and the various types of abnormal sperm forms recorded.

The data were analyzed for variance by the methods described by Snedecor (14).

RESULTS

The results of the examination of 879 ejaculates produced by 9 bulls between July 1, 1940, and August 31, 1941, are presented in this study.

Marked individual differences between bulls, and between successive weeks in the same bull, are apparent, and the significance of the variations has been tested. An analysis for variance on all samples collected from four bulls which were in the experiment for at least 12 consecutive months has been performed by the methods described by Snedecor (14). These bulls were normal, young, healthy animals with good breeding records and were maintained under the same system of management throughout the experiment:

Bull	*Age*	*Breed*
10A	1½ years	Holstein
Design	2 years	Jersey
Brampton	4 years	Jersey
Drummer	3 years	Ayrshire

A summary of the factors which were analyzed and the significant variations which were observed is presented in table 1. Significant differences between bulls occurred for each factor analyzed, and, as with other types of physiological studies on small numbers of farm animals, the importance of careful study and analysis of the data is apparent. Highly significant differences occurred between months for each factor except pH, and this showed significant variation at the 5 per cent level. These results suggest that seasonal trends in semen production and quality do occur. The bull-month interaction analysis (table 1) reveals that the concentration of spermatozoa, the numbers of abnormal sperm forms, the survival of spermatozoa, and the total motility rating show highly significant variations and that volume varied significantly at the 5 per cent level.

The results of an analysis designed to reveal the significant monthly variations from the mean of four bulls are presented in table 2. The data show that semen of consistently inferior quality, when compared with the mean, was produced during the months of July, August and September and that semen of superior quality was obtained during April, May and June.

Although it has been shown in table 2 that significant monthly variations in semen quality occurred (means of 4 bulls) these results do not necessarily indicate individual bull variations. Since too few samples were obtained each month from individual bulls to yield a satisfactory mean the twelve months of the year were grouped as follows: winter (January, February and March); spring (April, May and June); summer (July, August and September); fall (October, November and December). These divisions were made because no appreciable differences in semen quality occurred within the periods, and because these months are the most similar in average daily temperature and humidity.

TABLE 1

Summary of the analyses of variance of the semen characteristics of 4 bulls

Factor analyzed for variance	Source of variation		
	Between bulls	Between months	Bull-month interaction
Initial motility	++	++	–
Volume	++	++	+
Concentration of sperm/mm.³	++	++	++
Total sperm	++	++	–
pH	++	+	–
Abnormal sperm/1000	++	++	++
Survival period	++	++	++
Total motility rating	++	++	++

– = Non-significant.
+ = Significant.
++ = Highly significant.

Volume and initial motility. The seasonal trends of the average semen volume and initial motility of 9 bulls are shown graphically in figure 1. Seven of the 9 bulls produced spermatozoa with the lowest initial motility during the summer months. The variation of 2 of the bulls, Blend and Triumph, may be due to individual differences, although the fact that Blend was started in service in January, 1941, and was used for only limited service during the first 2 months, and that Triumph often exhibited little or no sex desire, may explain the differences observed. The average volume of semen per ejaculate did not show as much variation as initial motility and with one exception (Lad) the average semen volume was least in the summer months.

TABLE 2

Summary of the significant monthly variations of the semen characteristics of 4 bulls. *, †, ‡, §

Month	Initial motility		Volume		Conc. of sperm/mm.³		Total sperm		pH		Abnormal sperm		Survival period		Total motility rating	
	No.	Mean	No.	Mean	No.	Mean	No.	Mean	No.	Mean	No.	Mean	No.	Mean	No.	Mean
Jan.	31	4.26	31	3.78	31	804	31	2858	21	7.12 (++)	31	102.4 (+)	31	9.0	31	13.4
Feb.	34	4.21	34	3.42	34	864	34	2566	27	6.84	34	100.3 (+)	34	10.2 (++)	34	14.3 (+)
March	34	3.74	33	3.33	34	754	34	2741	21	6.89	34	119.9	34	9.1	34	12.4
April	43	4.16	43	4.13 (+)	43	1166 (++)	43	4948 (++)	40	6.86	43	104.9	43	10.1 (++)	43	14.7 (++)
May	41	4.29 (+)	39	3.45	39	1194 (++)	39	4224 (++)	34	6.90	39	118.7	39	9.1	39	15.5 (++)
June	43	4.35 (+)	43	3.61	43	1320 (++)	43	4576 (++)	38	6.83	43	103.3 (+)	43	8.9	43	13.2
July—'40 July—'41	63	3.65 (+)	63	2.76 (+)	61	866	61	2534	53	6.76	60	165.1 (++)	37	7.9	37	12.6
August—'40 August—'41	71	3.18 (++)	74	2.52 (++)	74	873	74	2473 (+)	70	6.85	62	165.4 (++)	74	4.4 (++)	74	7.4 (++)
Sept.—'40 Sept.—'41	62	3.55 (++)	60	2.72 (+)	60	760 (+)	60	2031 (++)	54	6.80	69	164.6 (++)	61	5.0 (++)	58	8.3 (++)
Oct.	42	4.55 (++)	42	3.63	42	693 (+)	42	2580	36	6.83	40	97.2 (++)	42	9.0	42	13.4
Nov.	35	3.89	35	2.71	35	777	35	2269	32	7.13 (++)	35	106.7	35	5.5 (++)	35	8.9 (++)
Dec.	38	4.50 (++)	38	3.57	38	932	38	3475	29	6.83	38	90.9	37	8.8	37	13.6
All months	537	3.94	536	3.21	534	914	534	3039	455	6.87	528	126.6	510	7.7	507	11.7

* + = Significant difference from mean.

† ++ = Highly significant difference from mean.

‡ Least significant mean difference $= t_{.05}\sqrt{VE\left(\frac{1}{N}+\frac{1}{N_2}\right)}$

§ Highly significant mean difference $= \frac{t_{.01}}{t_{.05}} \times$ least significant mean difference.

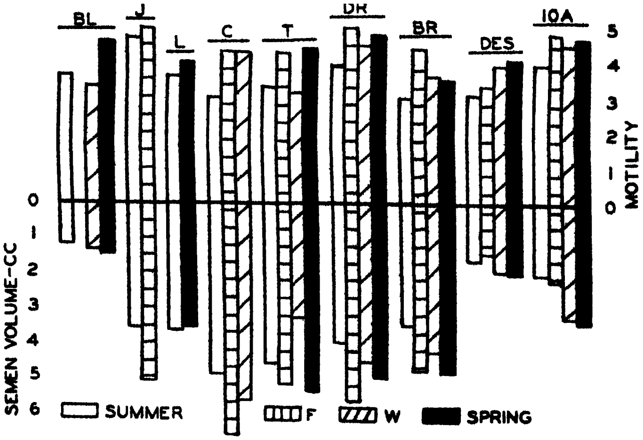

FIG. 1. The effect of season on the average semen volume and average initial sperm motility of individual bulls. Explanation: Bl—Blend; J—Jackie; L—Lad; C—Captain; T—Triumph; Dr—Drummer; Br—Brampton; Des—Design.

Concentration and total spermatozoa. The average number of sperm per cubic millimeter of semen was greater during the spring than in any other season, and the total number of spermatozoa per ejaculate showed the same trend (figure 2).

Hydrogen-ion concentration. As shown in figure 3 the average initial pH of the semen showed little seasonal change in either the same or between different bulls and there were no significant trends.

Abnormal spermatozoa. With the exception of Captain all bulls produced significantly greater numbers of abnormal sperm forms per thousand spermatozoa during the summer months than during any other season. The

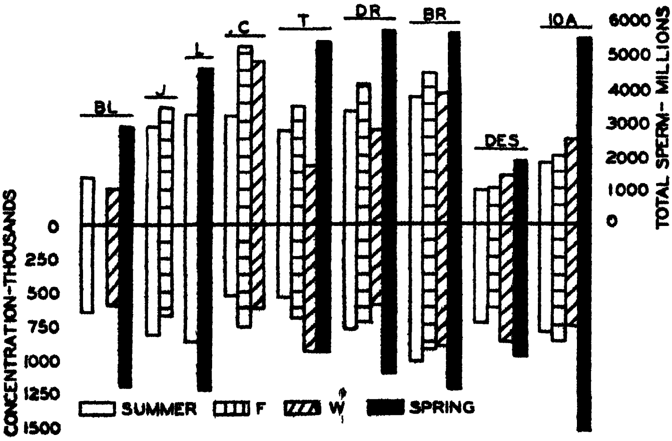

FIG. 2. The effect of season on the average sperm concentration and the average total sperm per ejaculate of individual bulls.

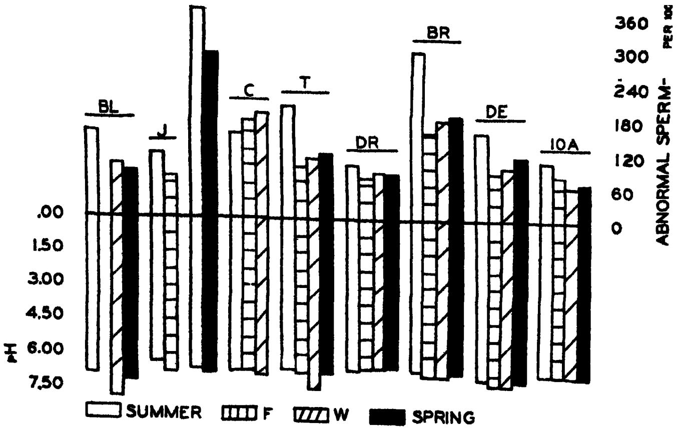

Fig. 3. The effect of season on the average pH and the average numbers of abnormal sperm per thousand spermatozoa of individual bulls.

numbers of abnormal sperm present in semen samples obtained during the fall, winter and spring were highly comparable (figure 3).

Survival period and total motility rating. As shown in figure 4 considerable individual variation was observed in the survival of spermatozoa and in the total motility rating. However, the survival and total motility ratings were usually poorest during the summer months when compared to the fall, winter and spring periods.

Summary of significant seasonal variations. When seasonal comparisons were made by computing the least significant mean differences of individual bulls for all methods of semen evaluation with the exception of pH, certain

TABLE 3

Summary of the significant seasonal variations of 9 individual bulls when all methods of semen evaluation except pH were considered

	Spring and summer		Fall and summer		Winter and summer		Spring and fall		Winter and fall		Spring and winter	
	Sp.	S.	F.	S.	W.	S.	Sp.	F.	W.	F.	Sp.	W.
Total number of comparisons	49	49	49	49	49	49	35	35	42	42	42	42
No. of highly significant comparisons showing production of superior quality semen	31	0	20	0	14	1	11	0	3	2	14	0
No. of significant comparisons showing production of superior quality semen	8	0	3	0	5	2	6	0	1	2	2	0
No. of non-significant comparisons	10	10	26	26	27	27	18	18	34	34	26	26

trends are evident. These data, which are summarized in table 3, show that when spring and summer semen quality were compared, spring semen was superior to that produced during the summer in 39 of 49 comparisons. Spring semen was equal to or superior to that produced during the fall and winter, and the semen of the poorest quality was produced during the summer months.

Temperature as a factor in seasonal spermatogenesis. Although temperature, humidity, and quantity and quality of light vary during the four seasons of the year and although the conditions during the same months of different years are often dissimilar, it is believed that temperature changes are at least partially responsible for the differences in semen quality which were observed.

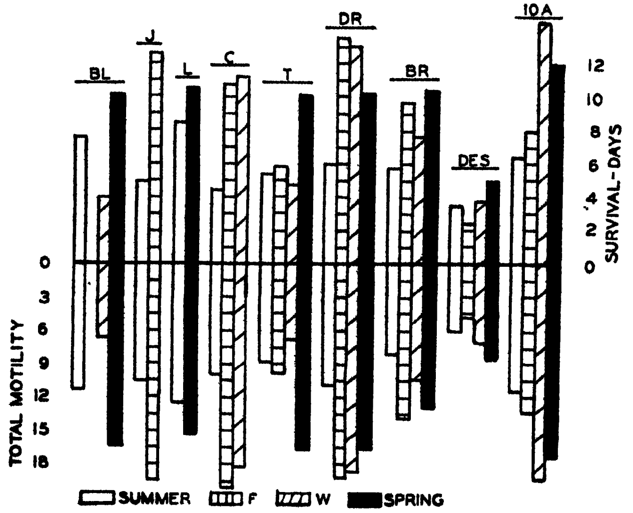

FIG. 4. The effect of season on the average total motility rating and the average survival period of spermatozoa of individual bulls.

The relationships between temperature and semen quality for the four bulls which were in the experiment for a complete year are presented in figure 5. Many of the trends in semen production follow the temperature curve rather closely. The average number of abnormal spermatozoa per thousand sperm increased more than 25 per cent during the months of July, August and September and total motility, survival, concentration, initial motility and volume were least at the time the maximum temperatures were observed. Rapid changes in temperature, either up or down, often seemed to be reflected in semen quality, and the fact that the average maximum temperature dropped 23 degrees Fahrenheit during November may have been at least partially responsible for the decreased quality of the semen which was collected during that month.

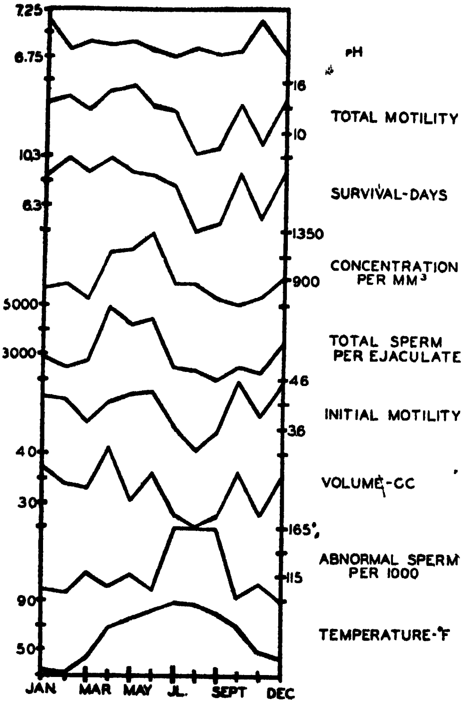

FIG. 5. Summary of the monthly variations in the semen quality of the 4 bulls, Drummer, Brampton, Design and 10A.

DISCUSSION

The causes of sterility and the factors which influence fertility are numerous and at best only partially understood. Sterility is usually easily recognized, for it is manifest by complete reproductive failure whereas fertility is a relative condition and must be expressed in relative terms. Those species which are strictly seasonal breeders are sterile during the non-breeding season, whereas such species as man, the domestic fowl, the horse, the bovine, and others, although classified as continuous breeders, tend to reproduce at different intensities throughout the year.

Although few critical studies of the influence of season upon reproduction in dairy cattle have been made, evidence has been accumulating that such influences do exist. Hammond (5) states that although cows will breed throughout the year the reproductive forces are at a maximum from May to July. Erb *et al.* (3) found breeding efficiency to be maximum during May and June and minimum during July and August, and Seath and Staples (13) of the Louisiana Station subsequently reported that more services per conception were required during the summer months than at any other period of the year.

A study of the reproductive efficiency of the Purdue Dairy Herd between 1920 and 1940 by Erb, Wilbur and Hilton (3) reveals an average breeding efficiency of 58.2 per cent in August as contrasted with 74.3 per cent in May. Although quantitative data were not available in the records of that 20-year period for determining the relative fertility of the males and females involved, it is likely that both sexes were affected. It is interesting to note that the quality of the semen produced by the bulls included in the present study, as determined by the laboratory methods described, was significantly superior during April, May and June and significantly inferior during July, August and September. Since the management and plane of nutrition of the bulls were similar throughout the experimental period it is concluded that the changes observed were the result of those factors which characterize the seasons—temperature, light, relative humidity and other general or obscure atmospheric factors.

Seasonal changes in semen quality of the bulls were not as striking as those which McKenzie and Berliner (8) have reported for Shropshire rams maintained under Missouri conditions. For example, the maximum average number of abnormal sperm forms per thousand spermatozoa was 165 in August in the bulls in contrast with more than 700 per thousand in Shropshire rams. Whereas the semen of this particular breed of rams might be considered relatively infertile during August, the semen produced by the bulls during the corresponding period might be described as of lowered quality when compared with the annual mean.

The experiment was not designed to determine the specific influences of temperature, light or humidity but it is believed that environmental temperature was undoubtedly of considerable importance. Many workers, as reviewed by Moore, (10) have demonstrated the deleterious effects of elevated temperatures upon spermatogenesis in mammals. The direct application of heat to the testis, the insulation of the scrotum, the replacement of the testes in the abdominal cavity, or a continued febrile condition, cause partial or complete inhibition of spermatogenesis. The increased rate of respiration and uneasiness which is characteristic of cattle and other farm animals, during periods of prolonged hot, humid weather is an indication that the regulation of body temperature is difficult and it would not be considered unusual if certain body processes such as semen production, were interfered with. The fact that McKenzie and Berliner (8) were able to decrease semen quality during the winter months by placing a group of rams in a warm, humid room is an indication that environment can exert a marked influence upon spermatogenesis in the normal intact male. Whether these effects are directly upon the testis, the hypophysis and/or other endocrines remain at present unexplained.

SUMMARY

1. Analyses of variance in various characteristics of semen production between bulls and between months reveal highly significant differences between bulls and between months for all factors studied except pH.

2. The average semen volume was least in July, August and September.

3. The average initial motility was least in July, August and September.

4. The average concentration of spermatozoa and total sperm per ejaculate was maximum during April, May and June.

5. The average period of sperm survival was least in August, and lower in July, September and November than during any other months.

6. The average number of abnormal spermatozoa was 25 per cent greater during July, August and September than during the next highest month of the year.

7. No significant seasonal variations in pH were observed.

8. The quality of the semen produced by the bulls in this study was significantly superior during the spring and significantly inferior during the summer. The semen produced during the fall and winter months did not vary significantly from the mean.

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A COMPARATIVE STUDY OF THE FROST LITTLE PLATE AND STANDARD PLATE METHODS FOR THE BACTERIOLOGICAL EXAMINATION OF MILK, CREAM, AND ICE CREAM*

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In 1915 Frost (1) presented a technique for making a rapid microscopic colony count of bacteria in milk, which has since been called the Frost little plate method. A detailed procedure was published by him in 1921 (5). Frost (2, 3, 4) and others (6, 8) have made comparative studies of the little plate and standard plating procedure and reported a good agreement between bacteria counts of milk obtained by these two procedures. However, considering the lack of any published reports, the little plate method for determining bacteria counts of milk appears to have been in disuse since 1922. Johns (7) adapted the method for making yeast and mold counts of butter.

The "old" standard nutrient agar was used in the above-mentioned studies. This medium was not a highly nutritive one; therefore Frost recommended a minimum three- or four-hour incubation period for milk in which the bacteria had been actively growing and a minimum eight- or nine-hour incubation period for both low-count milk and milk that had been recently pasteurized. In July of 1939 the American Public Health Association replaced the above agar with the more nutritive tryptone-glucose-extract-milk agar. This change and the fact that most milk plants, creameries, and health departments now have microscopes available, suggested the possibility that the Frost little plate method may be extremely valuable to milk plant operators and health officials in their milk control work insofar as the speed of determining the numbers of living bacteria in milk, cream, and ice cream is concerned. Accordingly, this study of the Frost little plate was made, employing the tryptone-glucose-extract agar to determine the bacteria count of milk, cream, and ice cream.

METHOD

Milk, cream, and ice cream samples were examined for bacteria count by both the Frost little plate (incubated in moist chamber at 37° C.) and

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* Editor's Note: Because of the present limited supply of agar for bacteriological purposes this report is of particular timely interest.

standard plate methods (48 hours of incubation at 37° C.). The milk samples included raw, pasteurized, homogenized, and chocolate milk. Both coffee and whipping cream were similarly checked. All types of ice cream were included in this study. The medium used in determining the standard plate count was the tryptone-glucose-extract-milk agar, while the same medium without adding skimmilk was used in the Frost little plate count.

RESULTS AND DISCUSSION

A. *Milk and cream.* In presenting his method, Frost suggested a variable incubation period depending upon the history of the sample, and that the little plates made from milk with a low count or recently pasteurized milk should be given a long incubation period while those made from milk in which the bacteria had been actively growing required only a short incubation time. This is highly unsatisfactory from a routine standpoint since it depends too greatly upon the judgment of the technician. This study was made, employing tryptone-glucose-extract agar, to determine the actual hours of incubation required of the Frost little plate to yield a count comparable with that obtained by the standard plate method.

The results of three raw and three pasteurized milk samples with both high and low bacteria counts are presented in table 1; these are representative of many samples examined. In each case, the four-hour period of incubation of the Frost little plates yielded similar counts to those obtained upon 48 hours of incubation in the standard plate procedure. The data

TABLE 1

A comparison of the bacteria counts obtained in three raw and three pasteurized milk samples upon various hours of incubation of the Frost little plate with the standard plate count

Hours of incubation of Frost little plates	Raw milk			Pasteurized milk		
	1	2	3	4	5	6
	Standard plate count per cc. of milk					
	5,500	20,000	480,000	500	5,000	40,000
	Frost little plate count per cc. of milk					
1	1,000	2,500	30,000	50	100	500
2	3,000	10,000	126,000	200	1,000	10,000
3	5,000	20,000	294,000	500	5,000	30,000
4	**7,000**	**18,000**	**400,000**	**600**	**6,000**	**42,000**
5	7,000	20,000	400,000	600	8,000	35,000
6	6,000	20,000	420,000	500	5,000	50,000
7	6,000	18,000	420,000	700	5,000	40,000
8	7,000	18,000	420,000	700	7,000	50,000
9	7,000	20,000	400,000	600	7,000	50,000
10	7,000	23,000	420,000	500	5,000	40,000
11	7,000	23,000	420,000	600	7,000	40,000
12	7,000	23,000	400,000	600	7,000	42,000
18	7,000	20,000	400,000	600	7,000	42,000
24	7,000	20,000	400,000	600	7,000	38,000

further show that the little plates may be incubated for periods as long as 24 hours without materially affecting the bacteria count. A four-hour period of incubation in a moist chamber of 37° C. appears to be sufficient incubation time for the Frost little plates in determining bacteria counts of milk and cream.

The presence of chocolate particles may be confusing to the inexperienced technician in the examination of some chocolate milk. This can be largely corrected by making a 1 to 10 or 1 to 100 dilution of the chocolate milk before preparing the little plates. The count obtained must then be multiplied by this dilution to obtain the total number of living bacteria present.

A series of 445 samples was checked for bacteria count by both the Frost little plate and the standard plate method. The data are summarized in table 2. Owing to the number of samples involved, the actual count of each sample is not given, but the bacteria counts obtained are grouped in convenient classes. In considering the classes selected it is obvious that a sample having a standard plate bacteria count of 9,000 and a count of 11,000 on the Frost little plate would be placed into two different classes. Yet from a practical standpoint the two bacteria counts compare very favorably one with the other. The bacteria counts obtained by the two methods compared favorably. This is indicated by the majority of samples belonging to the same bacteria-count class of both methods. The remaining few having slightly higher or slightly lower counts. In no case was there a marked difference in bacteria counts of a sample as determined by the Frost little plate and standard plate methods. In our opinion, the Frost little plate method will not replace the direct microscopic count in the examination of raw producer milk samples, but its use will result in a significant saving of time and materials in the determination of the number of living bacteria present in pasteurized milk and cream.

The bacteria counts of 10 samples of pasteurized milk, obtained by standard plate, Frost little plate, and the direct microscopic methods, are presented in table 3. Samples 1 to 5 inclusive yielded comparable low bacteria counts by all three methods, while samples 6 and 7 had high direct microscopic counts but low counts as determined by both cultural methods. Thermoduric bacteria were present in large numbers in samples 8 to 10 inclusive, as demonstrated by the close agreement of bacteria counts obtained by all three methods. Since both the living and dead bacteria may be stained and counted in the direct microscopic count of milk, this examination cannot be relied upon to give an accurate indication of the numbers of living bacteria in pasteurized milk. To determine the living bacterial content of pasteurized milk or cream, one of the cultural methods must be used. A saving of time will result if the Frost little plate method, with its four-hour incubation period, is used instead of the 48 hour incubation in the standard plate procedure.

TABLE 2

A summary of the standard plate count and Frost little plate count of 445 milk samples; 321 were raw producer milk samples while the remaining 124 included chocolate milk, raw and pasteurized milk, homogenized milk, and coffee and whipping cream

Standard count* plate		Frost little plate count*											
		Less than 1T	1T–10T	10T–25T	25T–50T	50T–75T	75T–100T	100T–250T	250T–500T	500T–750T	750T–1M	1M–2M	Over 2M
	Number	62	168	70	31	12	13	23	16	12	6	14	18
Less than 1T	55	31	18	6									
1T– 10T	165	28	124	13									
10T– 25T	81	3	25	42	11								
25T– 50T	21		1	6	11	3							
50T– 75T	27			3	7	7	7	3					
75T–100T	6				1		3	2					
100T–250T	23				1	2	3	15	1	1			
250T–500T	14							2	7	3	1	1	
500T–750T	13							1	7	2		2	1
750T–1M	5								1	1	3		
1M–2M	17									4	2	8	3
Over 2M	18									1		3	14

* T = thousand, M = million.

TABLE 3

A comparison of counts of pasteurized milk, both with and without thermoduric bacteria, obtained by the standard plate, Frost little plate, and direct microscopic methods

	Bacteria count per cc. of milk		
	Standard plate	Frost little plate	Direct microscopic (clump count)
	Thermoduric bacteria absent		
1	1,500	850	5,000
2	500	425	5,000
3	800	560	5,000
4	4,000	3,500	5,000
5	3,000	3,500	5,000
6	10,000	8,500	60,000
7	8,000	12,000	120,000
	Thermoduric bacteria present		
8	55,000	42,500	40,000
9	90,000	130,000	140,000
10	110,000	130,000	120,000

B. *Ice cream.* A comparison was made of the standard plate count of ice cream with the bacteria counts obtained upon varying periods of incubation of the Frost little plate. The results of six samples are presented in table 4 and indicate that an eight-hour minimum period of incubation of the Frost little plate yielded results that were essentially the same as the standard plate count. All Frost little plates were incubated eight hours for subsequent bacteria counts of ice cream. If not convenient to remove the little plates at eight hours, they may be incubated as long as 24 hours with-

TABLE 4

A comparison of the standard plate count of six ice cream samples with the counts obtained upon various hours of incubation of the Frost little plate

Hours of incubation of Frost little plates	1	2	3	4	5	6
	Standard plate count per gram of ice cream					
	200	350	4,800	350,000	630,000	10,000,000
	Frost little plate count per gram of ice cream					
4	50	200	300	200,000	400,000	6,000,000
5	50	250	500	200,000	480,000	8,000,000
6	50	350	800	300,000	450,000	8,000,000
7	50	350	1,800	400,000	600,000	10,000,000
8	**100**	**500**	**4,000**	**400,000**	**750,000**	**12,000,000**
9	100	400	4,000	350,000	700,000	10,000,000
10	150	500	5,000	350,000	700,000	10,000,000
11	100	500	4,000	400,000	650,000	12,000,000
12	200	450	6,000	400,000	700,000	10,000,000
13	200	500	4,500	400,000	700,000	10,000,000
14	100	400	4,000	400,000	700,000	10,000,000
15	150	550	6,000	400,000	700,000	12,000,000
20	150	450	6,000	350,000	800,000	12,000,000
24	100	400	4,000	350,000	700,000	10,000,000

out affecting the results. The colonies were of sufficient size after eight hours of incubation to be differentiated from strawberry seeds, nut fragments, and chocolate particles in these flavors of ice cream. The technician who is not familiar with the microscopic appearance of strawberry seeds, nut fragments and chocolate particles may experience some difficulty in differentiating between these particles and colonies. In case of doubt, the use of higher powered objectives will reveal individual bacteria along the edge of colonies. Ice creams containing particulate matter may be diluted 1 to 10 or 1 to 100 before preparing the Frost little plates, thus reducing the amount of foreign material in the little plate. In those cases, the Frost little plate count must be multiplied by the dilution made in order to obtain the total number of viable bacteria present.

One hundred and sixty-five samples of commercially prepared ice cream were checked for bacteria count by the Frost little plate and standard plate methods. A summary of the counts obtained is presented in table 5. A study of these data indicates that the counts obtained by the two methods compare very favorably. The Frost little plate may be substituted for the standard plate method for determining the living bacteria count of ice cream. Again, as in the case of milk, the saving of time in obtaining the little plate count is of decided practical importance to both ice cream manufacturers and health officials.

FROST LITTLE PLATE METHOD FOR MAKING A MICROSCOPIC COLONY COUNT OF MILK, CREAM, AND ICE CREAM

1. Place a test tube rack, containing enough sterile tubes for the number of samples to be tested, into a water bath at 45 to 50° C.

2. Place 0.5 cc. of milk into a test tube in the water bath.

    (a) In the examination of ice cream, weigh 0.5 gram into the sterile tubes in step 1 prior to placing the tubes into the water bath. Keep the tubes in a pan of ice water or a refrigerator while weighing the remaining samples.

    (b) When examining chocolate milk or strawberry, nut and chocolate ice cream, make a 1 to 10 or 1 to 100 dilution before proceeding with step 2, thus reducing the number of particles in the preparation. The final count must be multiplied by this dilution when made.

    (c) Do not allow more than 15 minutes between step 2 and completing step 4.

3. Add 0.5 cc. of sterile tryptone-glucose-extract agar, cooled to 50° C., into each tube in the water bath, and mix by shaking.

4. Using a sterile pipette place 0.1 cc. of the agar milk mixture onto a sterilized microscopic slide (sterilized in the naked flame) and spread evenly over an area of four square centimeters with the tip of the pipette. (Two or three such films can be put on the ordinary microscope slide.)

TABLE 5

A comparison of the standard plate count with the Frost little plate count of 165 ice cream samples

Standard plate count*		Frost little plate count per gram of ice cream											
		Less than 1T	1T–10T	10T–25T	25T–50T	50T–75T	75T–100T	100T–250T	250T–500T	500T–750T	750T–1M	1M–2M	Over 2M
	Number	11	71	32	14	10	1	8	10	3	0	2	3
Less than 1T	9	5	4										
1T– 10T	80	6	66	8									
10T– 25T	29		1	23	5								
25T– 50T	14				9	5							
50T– 75T	6			1		4	1						
75T–100T	3							3					
100T–250T	9					1		4	4				
250T–500T	8							1	6	1			
500T–750T	3									2		1	
750T–1M	1											1	
1M–2M	1												1
Over 2M	2												2

* T = thousand, M = million.

5. The little plate is allowed to harden and then placed into a moist chamber at 37° C. for a minimum of *four hours of incubation for milk and cream,* or a minimum of *eight hours of incubation for ice cream.* (A moist chamber can be made out of any container with a tight-fitting cover by filling the container approximately ¾ full of water. A wire platform must be built above the level of the water to hold the slides during incubation. The proper temperature of bath is maintained by leaving the moist chamber in the 37° C. incubator at all times.)

6. After incubation the plates are dried at a temperature slightly under 100° C. (Agar should not melt; plates should be dried rather slowly to prevent cracking the medium.)

7. Stain with a methylene blue solution. (To prepare add 10 cc. of a saturated alcoholic solution to 90 cc. of water.) This should stain the colonies deeply and leave a faint blue background.

8. Wash slides to remove excess stain being careful not to wash the film off the slides (best results are obtained by allowing the water to hit the reverse side of slide—enough water will flow over the preparation to remove the excess stain). Dry preparations.

9. Examination: Use lower power (if few colonies), high dry power or oil immersion (if many colonies) to determine the average number of colonies per field (Count *50 fields* when few colonies are present, and *10 fields* when many colonies are present.) and note bacteria count by referring to the table:

TABLE 6

FROST LITTLE PLATE COUNTS

(This table can be used with all miscroscopes having a factor of 300,000 in the direct microscopic counting of bacteria in milk)

	Colonies in fields	Bacteria per cc.		Colonies in fields	Bacteria per cc.		Colonies in fields	Bacteria per cc.
Low Power Objective	1 ... 50 =	50	High Dry Objective	1 ... 1.6 =	30,000	Oil Immersion Objective	2 ... 1 =	480,000
	1 ... 25 =	100		1 ... 1 =	42,000		3 ... 1 =	720,000
	1 ... 12.5 =	200		2 ... 1 =	84,000		4 ... 1 =	960,000
	1 ... 5 =	500		3 ... 1 =	126,000		5 ... 1 =	1,200,000
	1 ... 2.5 =	1,000		4 ... 1 =	168,000		6 ... 1 =	1,440,000
	1 ... 1 =	2,500		5 ... 1 =	210,000		7 ... 1 =	1,680,000
	2 ... 1 =	5,000		6 ... 1 =	252,000		8 ... 1 =	1,920,000
	4 ... 1 =	10,000		7 ... 1 =	294,000		9 ... 1 =	2,160,000
	6 ... 1 =	15,000		8 ... 1 =	336,000		10 ... 1 =	2,400,000
	8 ... 1 =	20,000		9 ... 1 =	378,000		15 ... 1 =	3,600,000
	10 ... 1 =	25,000		10 ... 1 =	420,000		20 ... 1 =	4,800,000

SUMMARY

The incubation of Frost little plates at 37° C. in a moist chamber for four hours yielded bacteria colony counts of milk and cream comparable to the standard plate count. The chocolate particles of some chocolate milk may confuse the technician unless a 1 to 10 or 1 to 100 dilution is made before preparing the little plate.

Comparable counts were obtained upon examination of ice cream by the standard plate and Frost little plate methods when the little plates were incubated for a minimum period of eight hours at 37° C. in a moist chamber. Ice cream containing seeds, nuts, or chocolate particles may be diluted as in the case of chocolate milk before preparing the little plates.

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STUDIES ON KETOSIS IN DAIRY CATTLE. I. EFFECT OF STALL AND PASTURE FEEDING UPON THE CONCENTRATION OF BLOOD AND URINARY ACETONE BODIES OF DAIRY CATTLE[1]

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The problem of ketosis is becoming of as great concern to dairymen as it has been to professional workers during recent years. The fact that the incidence of ketosis is greatest in late winter and early spring and that it usually occurs shortly after parturition when there is an increased demand upon the body for nutrients for milk production has led many to believe that the causative factor or factors are nutritional. Others believe that the condition is pathological. This view receives support from the fact that it has been observed to be present in toxic conditions such as metritis, pleuritis, and hemorrhagic septicemia. While some of the aspects of the physiology of ketosis in cattle have been studied and several possible causative factors have been suggested, the actual predisposing factors which bring about "spontaneous" ketosis in cattle are not known.

In a review of the available literature, a total of 126 analyses for total blood acetone bodies of apparently normal cattle (3, 6, 7, 10, 16, 17, 19, 20) averaged 2.99 mg. per cent acetone (min. 0.69 and max. 5.54), while 58 blood acetone body analyses of cows diagnosed as having ketosis (3, 7, 8, 9, 11, 14, 16, 17, 18, 19, 20) averaged 33.61 mg. per cent acetone (min. 2.40 and max. 89.30). Eighty-six analyses (3, 4, 5, 7, 10, 16, 17, 19, 20) for urinary acetone bodies of apparently normal cows averaged 10.00 mg. per cent acetone (min. 2.50 and max. 70.00) and 53 analyses for urinary acetone bodies of cows diagnosed as having ketosis (3, 4, 7, 8, 9, 14, 15, 16, 17, 18, 19, 20, 22) averaged 253.77 mg. per cent acetone (min. 10.00 and max. 1568.00).

Various studies have been carried out in recent years pertaining to the blood and urinary acetone bodies in several species because of the importance of these substances in both normal and abnormal metabolism.

The literature in reference to ketosis in ruminants has been reviewed by

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[2] The experimental data in this paper are taken from a thesis presented by C. B. Knodt in partial fulfillment of the requirements for the degree of Master of Science in Dairy Industry, University of Connecticut, Storrs Agricultural Experiment Station.

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Sampson and Hayden (18); Duncan, Huffman, and Tobin (7); Sampson and Boley (14) and others. For a review of the literature relating to the underlying mechanisms involved in the production of ketone bodies see Soskin and Levine (21).

It appeared that studies relative to the normal variations in the concentration of blood and urinary acetone bodies and their component fractions would give us some insight into the normal metabolism of ketone bodies in dairy cattle as well as some of the factors involved in the development of ketosis. Accordingly a rather comprehensive study was made of the normal variations in blood and urinary acetone bodies in the University dairy herd under the customary feeding regime.

EXPERIMENTAL

In the following experiments all blood samples were obtained from the jugular vein and potassium oxalate was used as the anticoagulant. The urine samples were obtained by massage of the vagina and were used only when a large amount of urine was excreted. All animals were apparently normal in every respect and care was taken to avoid excitation. The determinations were made immediately after the samples were obtained to minimize chemical changes which occur on standing. Blood and urinary acetone bodies were determined by the method of Barnes and Wick (1) and are expressed as mg. per cent acetone. β-hydroxybutyric acid was calculated by difference after determining the total acetone bodies and the fraction consisting of acetone and acetoacetic acid. Urine samples were diluted (1:1) with water before analyses to bring the concentration of acetone bodies closer to that of the blood acetone bodies and a corresponding correction was made in the calculation of the concentration of urinary acetone bodies.

Early in the course of our work considerable variation was observed in the concentration of the blood and urinary acetone bodies when consecutive samples were taken at short intervals during the day. As any considerable variation during the day in the level of the acetone bodies would be difficult to evaluate, an attempt was made to determine when the variations were greatest. The total blood and urinary acetone bodies were determined on samples drawn from two cows at intervals of two hours over a period of eight hours. In another experiment samples were drawn at two-hour intervals over a period of 24 hours and the fractions, acetone and acetoacetic acid, and β-hydroxybutyric acid were determined in addition to the total acetone bodies. In each of the three cases urine was drawn at 2:00 A.M. and discarded so that the samples drawn at 4:00 A.M. represented a two-hour excretion of urine. These cows received concentrates at 6:00 A.M., 2:00 P.M., and 10:00 P.M. Hay was fed daily at 10:30 P.M. and grass silage was fed twice daily at 8:15 A.M. and 2:30 P.M. The results are shown in table 1. Cows 1 and 2 showed an increase in total blood and urinary acetone bodies, which

TABLE 1

Variations in blood and urinary acetone bodies in mature cows at two-hour intervals (as mg. per cent acetone)

Cow no.	Time of day	Total blood acetone bodies	Total urinary acetone bodies
1	4: 00 A.M.	3.40	13.72
	6: 00 A.M.	3.15	9.37
	8: 00 A.M.	4.21	12.61
	10: 00 A.M.	5.02	19.97
	12: 00 M.	5.63	20.80
2	4: 00 A.M.	1.69	6.11
	6: 00 A.M.	1.34	5.93
	8: 00 A.M.	2.66	6.37
	10: 00 A.M.	3.27	8.00
	12: 00 M.	3.65	8.92

		Total acetone bodies		Acetone and acetoacetic acid		β-hydroxybutyric acid	
		Blood	Urine	Blood	Urine	Blood	Urine
3	2: 00 A.M.	2.18		0.36		1.82	
	4: 00 A.M.	2.46	17.74	0.61	6.14	1.85	11.60
	6: 00 A.M.	2.19	15.66	0.18	5.28	2.01	10.38
	8: 00 A.M.	2.25	16.52	0.63	4.96	1.62	11.56
	10: 00 A.M.	2.37	15.08	0.67	5.10	1.70	9.98
	12: 00 M.	3.82	17.50	0.82	4.80	3.00	12.70
	2: 00 P.M.	2.87	10.80	0.48	3.32	2.39	7.48
	4: 00 P.M.	1.46	11.90	0.72	2.62	0.74	9.28
	6: 00 P.M.	2.55	17.54	0.42	5.66	2.13	11.88
	8: 00 P.M.	2.33	18.00	0.51	6.84	1.82	11.16
	10: 00 P.M.	2.30	11.48	0.61	5.04	1.69	6.44
	12: 00 P.M.	2.14	12.32	0.30	4.28	1.84	8.04
	2: 00 A.M.	2.41	16.10	0.23	4.68	2.18	11.42

was especially marked at 10: 00 A.M. and 12: 00 M. In view of the data presented in figure 3, to be discussed later, it appears that these marked increases were the result of the feeding of grass silage at 8: 15 A.M. The smaller increases at 8: 00 A.M. can logically be attributed to the feeding of concentrates at 6: 00 A.M. on the basis of the same data. In the more detailed analyses in table 1 (Cow 3) the total blood and urinary acetone bodies were again high following the feeding of silage in the morning and were also high following the feeding of silage in the afternoon, although the effect was not as marked as in the other two cases. It will be observed that the larger variations in both blood and urinary total acetone bodies were usually due to variations in β-hydroxybutyric acid, the acetone and acetoacetic acid fraction usually accounting for only a small portion of the total change. Because of these observations all future samples were taken at 7: 00 A.M. in an attempt to avoid the larger fluctuations which appeared to occur later in the day.

One series of studies included 16 purebred cows composed of six Holsteins, five Guernseys, three Ayrshires, and two Jerseys which were used for the study of monthly variations in the concentration of total blood and urinary acetone bodies, acetone and acetoacetic acid, and β-hydroxybutyric acid. Five of these cows were dropped from the group during the 12-month period. Two were eliminated because of a tendency to become greatly excited during the drawing of the samples; one was sold from the herd; one calved prematurely and another was dropped from the group because of a severe edematous condition of the rear appendages prior to parturition.

The experimental period was begun on June 1, 1940, and samples were taken at monthly intervals to June 1, 1941.

All cows were on pasture daily from May 11, 1940, to October 12, 1940, and from May 2, 1941, to the completion of this work on June 1, 1941.

Three different commercial concentrate mixtures were fed but no variations in blood and urinary acetone bodies occurred which could be attributed to any of the three concentrate mixtures. These concentrate mixtures contained a large variety of grains and not less than 14 per cent crude protein.

Grass silage prepared by the addition of 80 pounds of molasses per ton of mixed grasses was the only silage fed. The cows received between 35 and 45 pounds of grass silage daily during the winter months, depending upon the weight of the animal. This amount was decreased by two-thirds when the cows went on early spring pasture and was gradually increased as summer progressed. Approximately the same amount of a fair quality of mixed grass hay, composed primarily of blue grass, was fed throughout the year.

The averages of the monthly analyses which were determined in a study of the normal variations in the concentration of blood and urinary acetone bodies are presented in figure 1. The initial samples were obtained in the month of June, 1940, after the cows had been on spring pasture for five to seven weeks. Both the blood and urinary acetone bodies were quite low at this time. There was an increase in the concentration of urinary acetone bodies and the component fractions, especially β-hydroxybutyric acid, as summer advanced. The concentration of blood acetone bodies was more variable, but a higher concentration of total blood acetone bodies was found during the months of September and October as compared to the month of June, 1940. A marked decrease in the concentration of blood and urinary acetone bodies and the component fractions was observed in November, 1940. A continual increase was observed in the concentration of blood and urinary acetone bodies beginning with the month of December, 1940, and continuing through March, 1941. Throughout the entire period of 12 months the increases and decreases in the total acetone bodies of blood and urine were accounted for primarily by changes in the concentration of β-hydroxybutyric acid. During the stall feeding period the rise in the fraction consisting of acetone and acetoacetic acid was also very marked in both blood and urine, although the increase was not as great as the increase in β-hydroxybutyric acid. A large decrease occurred in May, 1941, in the concentration of total blood and urinary acetone bodies and of the component fractions two to three weeks after the cows had been on pasture.

The concentration of total blood and urinary acetone bodies of the cows decreased quite rapidly after the first day of pasture feeding as shown in table 2, with the former decreasing somewhat more rapidly on a percentage basis than the latter. At the time the cows were placed on pasture the hay and grain feeding was continued at approximately the same level but the amount of grass silage fed was decreased by about two-thirds. It is of interest to note that the maximum decrease in acetone and acetoacetic acid in both blood and urine occurred after only one day of grass feeding. The blood and urinary β-hydroxybutyric acid on the other hand declined more gradually over a longer period of time, the decrease in total blood and urinary

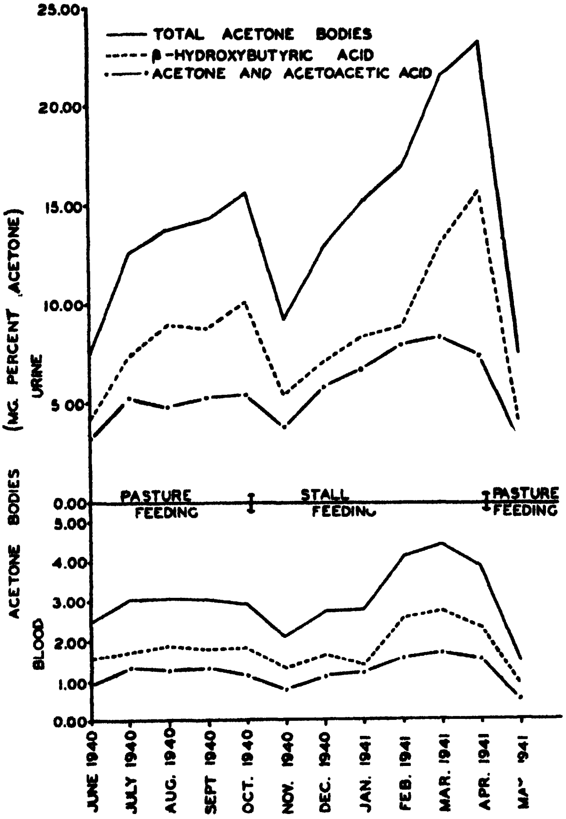

FIG. 1. Blood and urinary acetone bodies of mature cows on stall and pasture feeding. (Monthly analysis of 11 mature cows.)

acetone bodies after the first day of pasture feeding being due almost entirely to a decrease in β-hydroxybutyric acid.

Figure 2 presents the analyses for blood and urinary acetone bodies of a six-year-old Jersey bull at monthly intervals for a one-year period. In stall feeding the bull received a grain ration similar to that received by the herd cows in addition to approximately 10 pounds of grass silage per day and a fair quality of mixed grass hay. The bull was on pasture daily in the first summer to October 12, 1940, and after April 26, 1941, to the end of the experimental period. The lowest concentrations of blood and urinary acetone bodies and component fractions were observed during spring and early summer pasture and the highest concentrations were found in the April, 1941, analyses after six months of stall feeding. These observations are quite similar to those made on the cows during this same period.

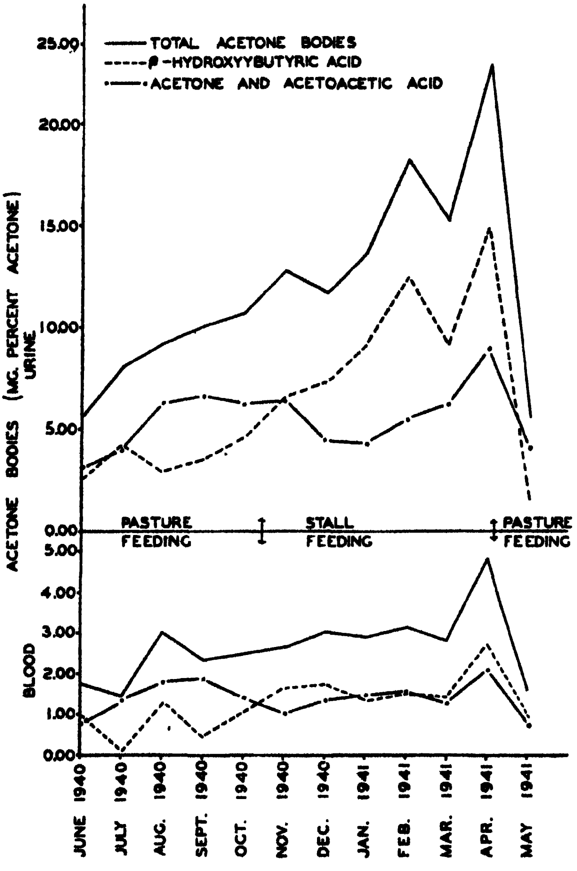

Fig. 2. Blood and urinary acetone bodies of a mature bull on stall and pasture feeding.

TABLE 2

Blood and urinary acetone bodies of 6 mature dairy cows on pasture feeding following 7 months of stall feeding

Date of analysis	Acetone bodies (expressed as mg. per cent acetone)					
	Acetone and acetoacetic acid		Total acetone bodies		β hydroxybutyric acid	
	Blood	Urine	Blood	Urine	Blood	Urine
5- 2-41	0.98	4.70	2.71	11.10	1.73	6.40
5- 3-41	0.32	2.79	1.98	9.88	1.66	7.09
5- 4-41	0.48	3.13	1.59	8.91	1.11	5.78
5-10-41	0.51	3.10	1.60	7.92	1.09	4.82
5-18-41	0.43	2.55	1.28	5.38	0.85	2.83

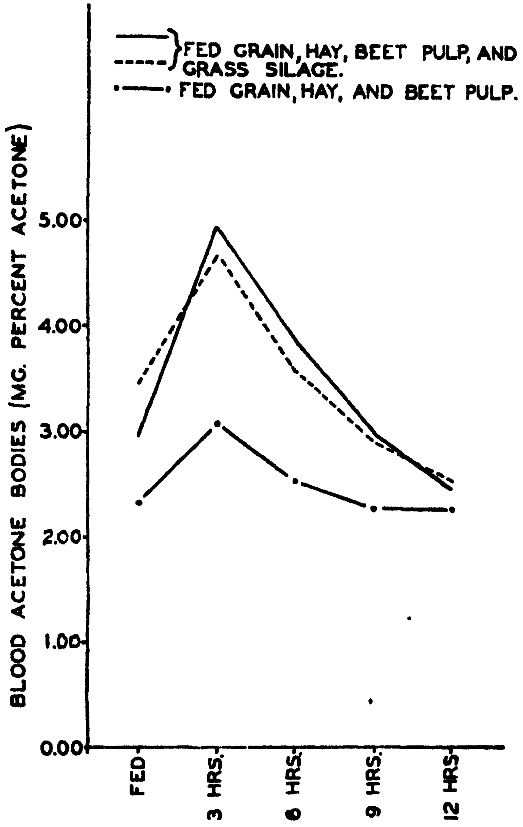

FIG. 3. Effect of grass silage feeding upon the blood acetone bodies of 3 mature cows.

To ascertain the immediate effect of silage feeding upon the concentration of blood acetone bodies, the experiment outlined below was conducted. Three cows which had not received any feed after 10:00 P.M. of the previous evening were fed the following feeds between 5:30 A.M. and 6:30 A.M. on the following morning: two cows received grain, beet pulp, fair quality mixed grass hay, and approximately 15 pounds of grass silage; one cow received the same ration with the exclusion of grass silage. No feed was received thereafter by these animals for 12 hours and blood samples were drawn at three-hour intervals beginning prior to the time of feeding and extending over a period of 12 hours. The variations in the concentration of blood acetone bodies over the 12-hour period are presented in figure 3. There was a rise in the acetone bodies of all three cows two hours after feeding followed by a rather marked decrease, approaching the previous level, after a period of nine hours. The cows receiving grass silage showed a much more marked increase in the blood acetone bodies, indicating that grass silage feeding

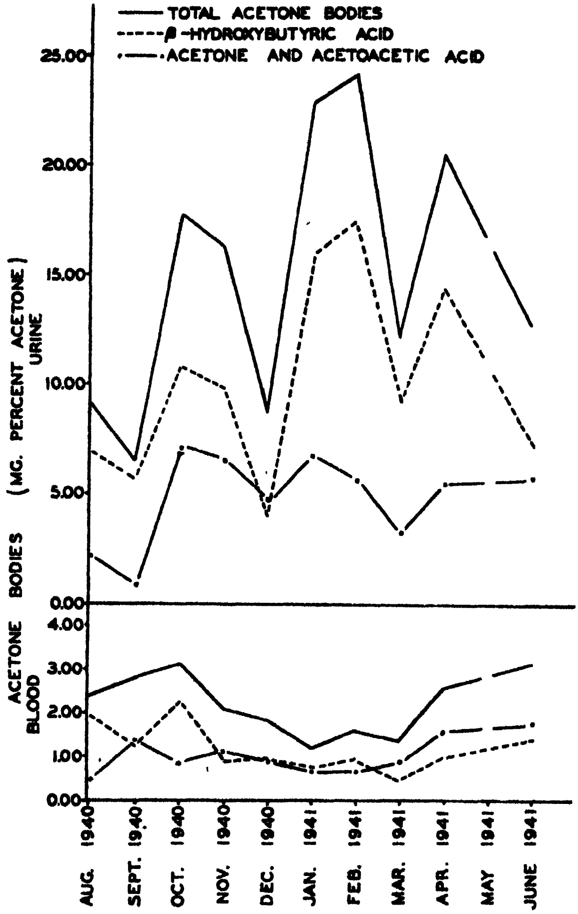

FIG. 4. Blood and urinary acetone bodies of 4 heifers 16 to 20 months of age as of August 1, 1940.

produced a much greater increase in the blood acetone bodies than grain and hay feeding alone. The effect of feeding in this experiment was more clear-cut than that shown in figure 1, probably because other feeds were not given during the day as in the previous experiment.

Data are presented in figure 4 on four heifers ranging from 16 to 20 months of age at the beginning of the experiment which extended essentially over the same period as that of the cows and the mature bull. These heifers were in pasture lots the year around and did not receive any additional feed during the summer months. Very little grass was available between October and April, however, and during this period the ration consisted entirely of a fair quality of mixed grass hay. The urinary values were extremely variable and presented no definite trend. The acetone bodies in the blood indicated a trend in which, surprisingly, the low values were observed during

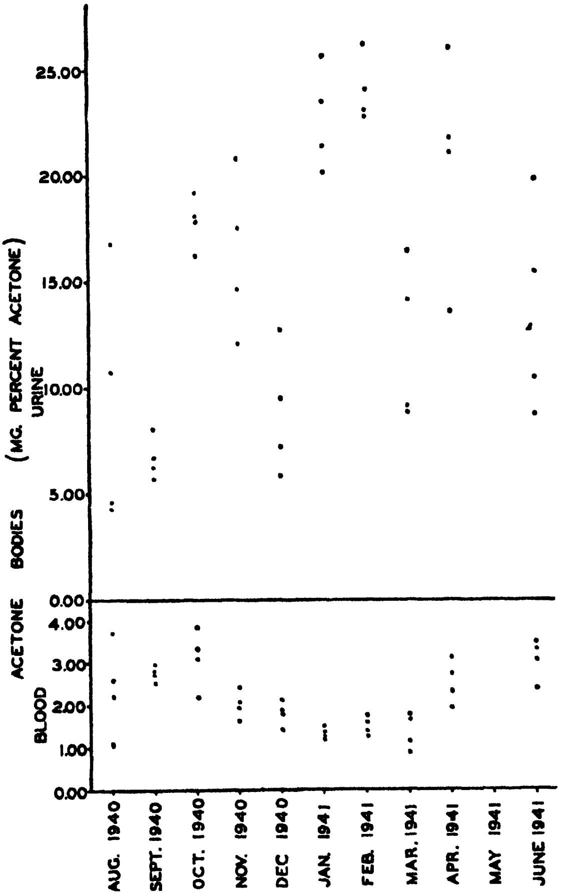

FIG. 5. Total blood and urinary acetone bodies of 4 heifers 16 to 20 months of age as of August 1, 1940.

the winter period on hay feeding while the high values occurred during the grass feeding period. As was observed in the mature cows and the aged bull, the marked variations in the urinary acetone bodies were due primarily to β-hydroxybutyric acid.

The scatter diagram of the total blood and urinary acetone bodies of this group of heifers is presented in figure 5. While the urinary acetone bodies were extremely variable, the total blood acetone bodies showed much less dispersion.

DISCUSSION

The variations observed in the blood and urinary acetone bodies of the mature cows and aged bull appear to be rather closely associated with pasture and silage feeding. It has been observed by Brouwer and Dijkstra (4) that when large quantities of silage of the butyric acid type were fed there were marked increases in the urinary acetone bodies. McAuliffe, Stone and Bechdel (13) reported an increase in volatile acids over a period of time in alfalfa silage prepared by the addition of molasses. Bender, Bosshardt, and Garrett (2) reported an increase in acetic acid, from 0.12 to 1.18 per cent, over a period of 35 days following ensilation. Most of this increase was observed during the first 10 days. The largest increase in the blood and urinary acetone bodies occurred in the cows and aged bull on stall feeding when the animals were receiving silage in which similar fermentations had probably taken place. In the mature cows low values were observed in June of 1940 and May of 1941, as well as in November of 1940. These periods correspond to spring and early summer pasture feeding and the feeding of fresh silage made from third crop alfalfa hay respectively.

The increases all occurred after the silage was at least several weeks old. This is of particular interest since it has been shown by MacKay, *et al.* (12) that the feeding of acetic acid to a phlorhizinized dog and fasting rats increased the acetone body production. The marked decrease in May 1941, which occurred after the cows were on grass was undoubtedly due in part to the decrease in the feeding of grass silage at this time. However, the fact that the acetone bodies continued to decrease over a 16-day period to such low values indicates that grass may have had an additional effect beyond that produced by the lowered level of silage feeding.

That the increase in the acetone bodies of the cows during the stall feeding period was more closely associated with the feeding of grass silage than any other single factor is borne out by the data, in figures 4 and 5, on four heifers which had not received silage at any time during the year. The lowest values were observed on these heifers during the winter months when the ration consisted solely of fair quality hay. The principal difference between these two groups in the blood acetone bodies was the high concentration of β-hydroxybutyric acid in the cows during the period of aged grass silage feeding. It is, indeed, puzzling that the blood acetone bodies of the heifers should have been so much lower on a ration of mixed grass hay than on pasture grass. The hay may have supplied less ketogenic material than the grass or the rumen flora may have been changed to such an extent that smaller quantities of acetone body precursors were formed in the rumen. This appears to be contrary to the data indicating that pasture grass produced a decline in the acetone bodies of the mature cows below that to be expected from the decline in silage intake.

As the level of acetone bodies in other species has been shown to be markedly affected by variations in energy intake this aspect must also be given consideration in any suggested explanation of the cause of the variations observed in these experiments. However, the variations observed do not appear to be associated with the relative energy intake. The milking cows which were rather heavily fed exhibited much greater concentrations of acetone bodies than the heifers which were maintained at a rather low nutritive level on mixed grass hay. This is further borne out by the data on the bull which was fed at a higher nutritive level than the heifers. The fact that the blood acetone bodies of the heifers were higher when the animals were on grass than on hay also suggests that rumen digestion may play an important part in the production of acetone bodies in the bovine.

The acetone and acetoacetic acid is relatively much more constant than the β-hydroxybutyric acid. This may indicate that ketone bodies are formed either directly or indirectly as the result of digestion in the rumen in which β-hydroxybutyric acid is the chief component. At least a part of the ketone bodies in the blood and urine of the bovine may be expected to have an endogenous origin and may account for the greater constancy of acetone and acetoacetic acid, assuming that ketone bodies, consisting predominantly of β-hydroxybutyric acid, are formed from ketogenic substances in the feed and/or ketogenic substances formed as the result of rumen digestion.

Although the increase in the blood and urinary acetone bodies during the stall feeding period was undoubtedly due primarily to silage feeding, it cannot be concluded with certainty that other factors were not involved. The fact that there was a continued increase throughout the entire stall feeding period suggests that other factors may have been involved. The data on the two-year-old heifers and the mature bull, however, indicate that the increased ketonemia and ketonuria in the cow group may have been due entirely to the ketogenic effect of silage.

The ketogenic effect of silage should not be misconstrued as indicating a relationship between grass silage feeding and clinical ketosis. In fact, it may well be that grass silage supplies nutritional substances needed for the prevention of clinical ketosis, as recent work (unpublished) indicates that pasture grass has a marked antiketogenic effect in cases of clinical ketosis.

SUMMARY

A marked increase in the concentration of total blood and urinary acetone bodies and their component fractions of acetone and acetoacetic acid, and β-hydroxybutyric acid was observed during the late winter and early spring months with animals receiving molasses-treated grass silage. The lowest concentrations of these substances were observed in these animals when they were on early spring and summer pastures and were receiving grain, hay, and only a relatively small quantity of silage.

It is believed that the relatively high values obtained for the group as a whole during the winter feeding period were due primarily to grass silage feeding. Although acetone and acetoacetic acid, and β-hydroxybutyric acid increased, the latter fraction was responsible for most of the rise. Since the feeding of fresh silage did not produce this effect, it appears that the increase in the acetone bodies on silage feeding was associated with the aging of the silage. In the light of the work of Bender *et al.* (2) and MacKay *et al.* (12) it is suggested that the increased production of the acetone bodies due to the feeding of aged molasses-treated grass silage is due primarily to the volatile acids in the silage.

Frequent observations on the blood and urinary acetone bodies of six cows showed a sharp decline in these substances immediately after the animals were placed on spring pasture. The blood and urinary acetone bodies had decreased 52.8 per cent and 51.5 per cent respectively after 16 days of daily pasture feeding. The maximum decreases in acetone and actoacetic acid were observed following the first day of pasture feeding. Blood and urinary β-hydroxybutyric acid, however, continued to decline throughout the 16-day period of observation. The sharp decline in acetone bodies after one day of pasture feeding was undoubtedly due primarily to the decreased silage feeding. The pasture grass feeding is believed to be partially responsible for the further continuous decline in the acetone bodies.

The blood acetone body levels of four heifers which were pasture fed during the summer months and received only mixed grass hay of a fair quality during the winter months were much lower while on hay feeding than on pasture feeding.

The blood acetone bodies increased following feeding, reaching a peak three hours after feeding, and then gradually decreased, returning to the pre-feeding level within nine hours. This effect was much more evident when aged grass silage was included in the ration.

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STUDIES ON KETOSIS IN DAIRY CATTLE. II. BLOOD AND URINARY ACETONE BODIES OF DAIRY CATTLE IN RELATION TO PARTURITION, LACTATION, GESTATION, AND BREED[1]

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A large percentage of the clinical cases of ketosis in dairy cattle occur during the first few weeks following parturition. This condition is associated with loss of appetite, a decrease in body weight, and a marked decline in milk production. Sjollema (10) early observed that ketosis frequently occurs during the first week to 10 days following parturition. Boddie (2) reported that ketosis usually occurs during the first six weeks following parturition and is frequently observed about two weeks after parturition. Duncan, Huffman, and Tobin (4) observed that the symptoms of ketosis were most marked two to six weeks following parturition in a herd of purebred Jersey cattle with a high incidence of ketosis.

Because of the large number of cases of ketosis observed shortly after parturition, emphasis was placed upon this particular period in the study of the normal level of the acetone bodies. Studies were also made of the effect of lactation, gestation, complete cessation of milking, and of breed upon the concentration of these substances in the blood and urine of dairy cows.

EXPERIMENTAL

During the course of this work the same precautions were observed in obtaining and analyzing the samples as were followed in the previous work (5). The method of Barnes and Wick (1) was used in the analyses of the acetone bodies.

Blood and urinary acetone bodies in relation to parturition and stage of lactation. In connection with the studies on the relationship of stall and pasture feeding to the level of blood and urinary acetone bodies (5), observations were also made of the levels of blood and urinary acetone bodies prior to and following parturition.

The data on 11 cows over a one-year period were plotted at 30-day intervals according to stage of lactation as shown in figure 1. There was an upward trend in the total urinary acetone bodies for a period of approximately

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[2] The experimental data in this paper are taken from a thesis presented by C. B. Knodt in partial fulfillment of the requirements for the degree of Master of Science in Dairy Industry, University of Connecticut, Storrs Agricultural Experiment Station.

90 days after parturition, followed by a gradual decline over the rest of the period. The total blood acetone bodies presented somewhat the same picture, although the trend was not as marked. A critical study of the data indicated that most of the rise during the first few months following parturition was due to the effect of grass silage feeding which results in a marked increase in blood and urinary acetone bodies (3).

Daily observations were made upon five of these cows for a period of three days prior to and including the day of parturition and for four days following parturition. These data indicate a rather marked decrease in urinary acetone bodies during the four-day period following parturition. There also appeared to be a decline in blood acetone bodies, especially during the first two days following parturition. An analysis of variance of these data showed a highly significant (F = 22.05) decrease in total acetone bodies, and a significant (F = 14.34) decrease in urinary β-hydroxybutyric acid, but the average decrease in urinary acetone and acetoacetic acid was not significant (F = 5.96). A similar analysis was made on the blood acetone bodies and it was found that the apparent average decline during the four-day period following parturition was not statistically significant, the

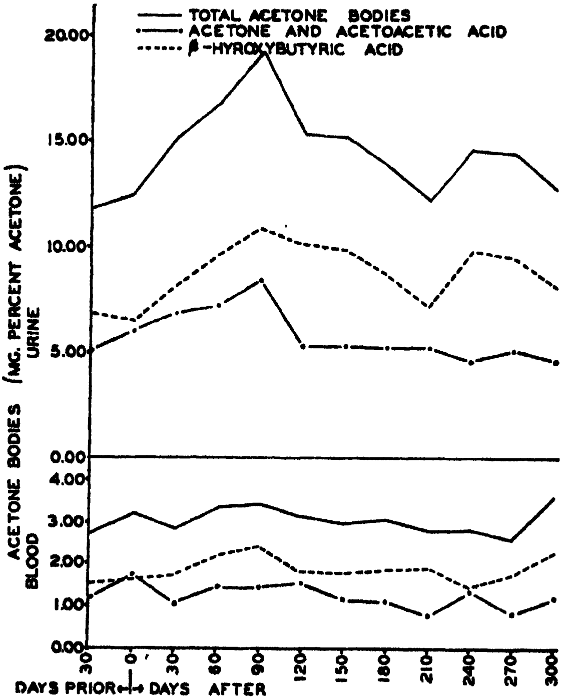

FIG. 1. Blood and urinary acetone bodies as related to stage of lactation. (Monthly analysis of 11 mature cows.)

F values being as follows: total acetone bodies (F = 2.48), acetone and acetoacetic acid (F = 0.64), and β-hydroxybutyric acid (F = 1.18).

Intensive studies upon the levels of blood and urinary acetone bodies of several cows prior to and following parturition showed large day-to-day variations. The results of such observations upon two cows are presented in figure 2. The day-to-day changes in blood and urinary acetone bodies are very large and while factors associated with parturition appear to affect the acetone bodies in certain cases any trend may be obviated by these variations.

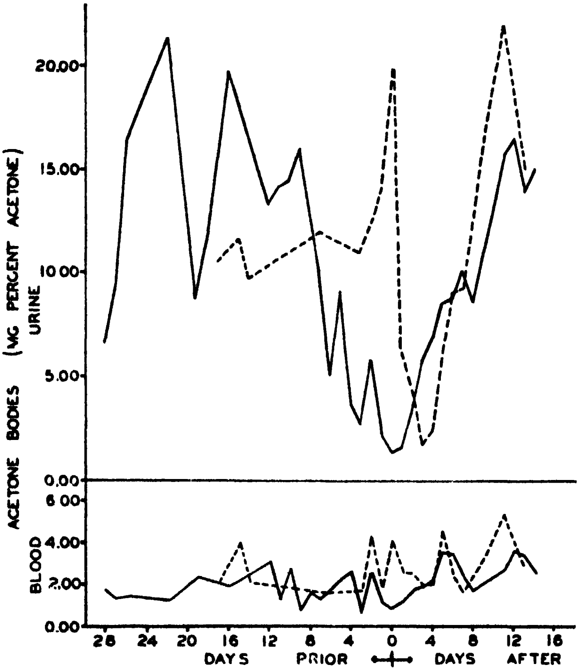

FIG. 2. Total blood and urinary acetone bodies of two mature cows prior to and following parturition.

The variations observed in the concentration of acetone bodies throughout the day at intervals of from two to four hours also showed large variations (5) although the magnitude of these changes was not quite as great as that observed prior to and following parturition. Although an attempt was made to continue the feeding of grass silage at the same level through the period of parturition, there were some variations in the consumption of silage during this period. While there may be a decrease over a relatively short period of time following parturition in some cases, it is deemed more significant that there is not a marked increase in blood and urinary acetone bodies during the period following parturition when the incidence of ketosis is greatest.

Blood and urinary acetone bodies during gestation. Blood and urinary analyses upon 10 cows at various stages of pregnancy are presented in figure 3. These data are quite irregular and indicate that factors other than pregnancy were affecting the concentration of blood and urinary acetone bodies. Undoubtedly some of the irregularity as well as the particular lack of an apparent trend is due primarily to normal day-to-day variations and to changes in feeding conditions.

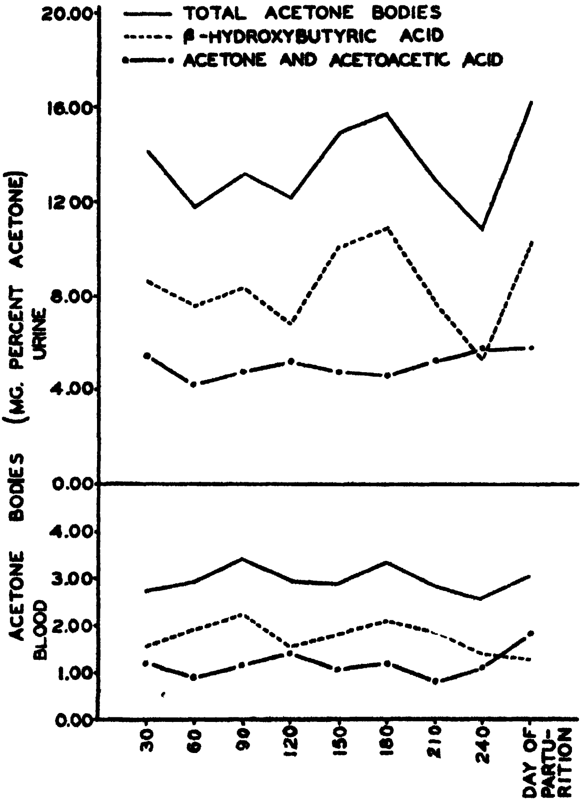

FIG. 3. Blood and urinary acetone bodies during gestation. (Monthly analysis of 10 mature cows.)

Blood and urinary acetone bodies prior to and following the complete cessation of milking. The utilization of β-hydroxybutyric acid by the lactating mammary gland was demonstrated by Shaw and Knodt (8). On the basis of our calculations a cow producing 20 kilos of milk per day must mobilize approximately 237.0 grams of β-hydroxybutyric acid per day for milk secretion. Because of this relatively large mobilization of acetone bodies for milk secretion purposes, a study was made to observe the effect of sudden cessation of milking upon the concentration of the acetone bodies in the blood and urine.

Five cows were studied for several hours prior to and following the complete cessation of milking. Normally these cows were milked at eight-hour intervals. It will be observed in figure 4 that there was no significant change in blood and urinary β-hydroxybutyric acid attributable to other than normal daily and hourly variations. The failure to observe an increase in the blood and urinary acetone bodies following the complete cessation of milking may be due to two factors: 1) that the gland continues to use β-hydroxybutyric acid for some time as in the case of glucose and amino acids (9), and 2) that there is an increased utilization of β-hydroxybutyric acid by the other tissues of the body.

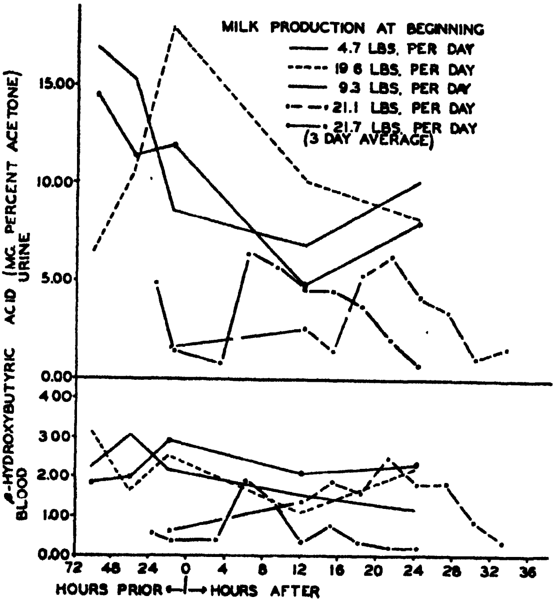

FIG. 4. Blood and urinary β-hydroxybutyric acid prior to and following the last milking.

Blood and urinary acetone bodies as related to breed. In the study of clinical ketosis at this station most of the cases have occurred in Jersey and Guernsey herds. The fact that more fat must be mobilized per unit of volume of milk produced by the cows of the higher testing breeds than those of the lower testing breeds suggests that the level of acetone bodies may be affected by breed. Since the production of acetone bodies by the liver may be expected to be proportional to the amount of fat catabolized, the cows were divided into two groups for purposes of comparison, in which one group was composed of four Guernseys and one Jersey and the other group was composed of four Holsteins and two Ayrshires. The data in figure 5 do not indicate any significant difference between the two groups during the spring and summer months. During the winter months the acetone bodies of the higher testing breeds were higher than those of the lower testing breeds. It appears that the difference may have been due to a somewhat higher level of grass silage feeding per unit of body weight to the higher testing group. The production of acetone bodies due to the feeding of grass silage probably accounted for such a large proportion of the total acetone body production that any differences due to breed were obviated.

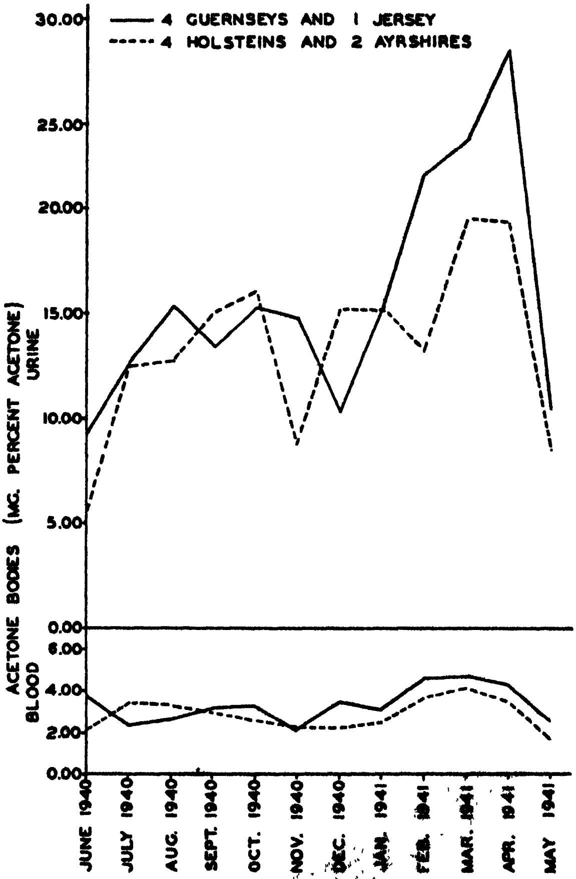

FIG. 5. Blood and urinary acetone bodies as related to breed and stall and pasture feeding.

Comparison of blood, milk, and urinary acetone bodies. Sampson and Boley (7) have reported the analyses of milk from six cows following recovery from ketosis in which they were unable to detect the presence of acetone bodies by the method of Van Slyke (6). Duncan, Huffman, and Tobin (4) presented data on the concentration of acetone bodies in the milk of cows during recovery from ketosis. They were unable to detect acetone bodies in the milk of several cows which appeared to have recovered from ketosis. Since we have shown that β-hydroxybutyric acid is used in substantial quantities by the mammary gland (8), the question arose as to whether all of the β-hydroxybutyric acid taken up by the gland is completely metabolized in the gland as in the case of blood glucose or whether some passes into the milk.

Analyses of 16 samples of blood, milk, and urine taken simultaneously are presented in table 1. These samples were analyzed for total acetone bodies and the component fractions of acetone and acetoacetic acid and of β-hydroxybutyric acid. Both β-hydroxybutyric acid and acetone and acetoacetic acid were found in all the milk samples, although three samples contained only traces of the former. Although the level of β-hydroxybutyric acid was higher in the blood than that of acetone and acetoacetic acid, there was a lower concentration of this substance in the milk. This might be expected on the basis of previous work which indicated that β-hydroxybutyric acid is taken up by the active gland while the fraction composed of acetone and acetoacetic acid is not used. The concentration of acetone bodies in these 16 samples of milk was as follows: 1.80 ± 0.88 mg. per cent total acetone bodies; 1.00 ± 0.63 mg. per cent acetone and acetoacetic acid; and 0.80 ± 0.63 mg. per cent β-hydroxybutyric acid. Although the concentration of acetone bodies in milk was usually lower than that in blood, there were four cases where these were higher. There did not appear to be any correlation between the milk and blood acetone bodies within the normal range of these values.

TABLE 1

Blood, milk and urinary acetone bodies of mature dairy cows (16 simultaneously drawn samples)*

	Total acetone bodies	Acetone and acetoacetic acid	β-hydroxybutyric acid
Blood	3.14 ± 1.23†	1.17 ± 0.63	1.97 ± 1.25
Milk	1.80 ± 0.88	1.00 ± 0.63	0.80 ± 0.63
Urine	12.09 ± 6.09	4.24 ± 2.95	7.85 ± 3.87

* Expressed as mg. per cent acetone.
† Standard deviation.

A comparison of blood and urinary acetone bodies of mature dairy cows. In the course of the various studies of the normal level of blood and urinary acetone bodies, extending over a period of 15 months, a total of 427 samples of blood and urine were collected simultaneously.

The mean concentrations of the blood and urinary acetone bodies and the extreme ranges observed are presented in table 2. The average concentrations of total blood and urinary acetone bodies were 2.66 ± 1.23 and 11.81 ± 6.56 mg. per cent acetone respectively. β-hydroxybutyric acid made up 59.39 per cent of the total blood acetone bodies and 61.73 per cent of the total

TABLE 2

Blood and urinary acetone bodies of mature dairy cows (427 simultaneously drawn samples from 16 cows)*

	Mean	Standard deviation	Min.	Max.
Total blood acetone bodies	2.66	1.23	0.31	6.95
Blood acetone and acetoacetic acid	1.08	0.66	0.12	5.62
Blood β-hydroxybutyric acid	1.58	1.00	0.06	5.52
Total urinary acetone bodies	11.81	6.56	0.61	34.26
Urinary acetone and acetoacetic acid	4.52	2.85	0.04	19.78
Urinary β-hydroxybutyric acid	7.29	4.78	0.04	23.82

* Expressed as mg. per cent acetone.

urinary acetone bodies. No evidence of clinical ketosis or of other abnormalities were observed in these animals during the experimental period.

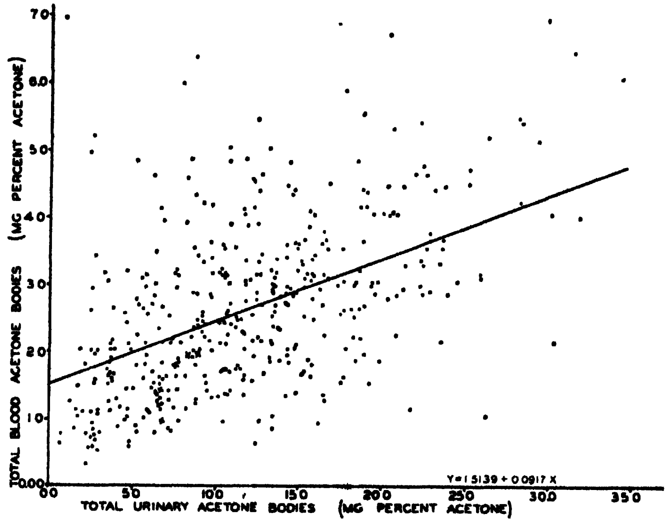

FIG. 6. Comparison of blood and urinary acetone bodies. (×= two separate values falling on the same point.)

The data presented in figures 6, 7, and 8 show a highly significant coefficient of correlation between the following blood and urinary substances: total acetone bodies (r = 0.5115), acetone and acetoacetic acid (r = 0.3315), and β-hydroxybutyric acid (r = 0.3750). It will be noted in figures 6, 7, and 8 that, although there was a significant correlation between the blood and urinary concentrations of these substances, there was a very large dispersion about the regression line.

There were highly significant coefficients of correlation between the total blood acetone bodies and the component fractions of acetone and acetoacetic acid (r = 0.5920), and β-hydroxybutyric acid (r = 0.8377). Simi-

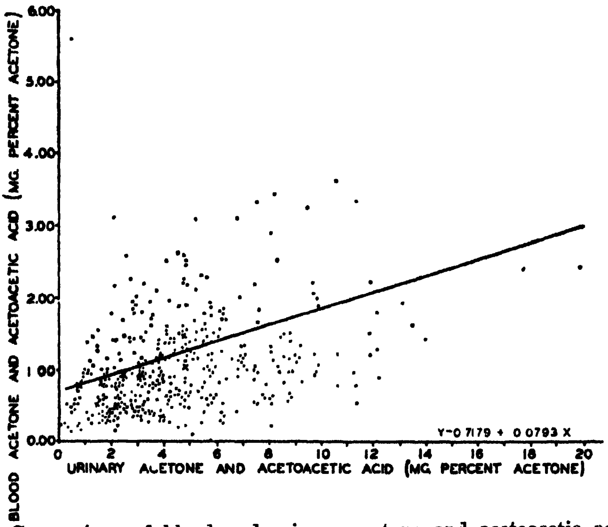

FIG. 7. Comparison of blood and urinary acetone and acetoacetic acid. (× = two separate values falling on the same point.)

larly there were significant coefficients of correlation between the total urinary acetone bodies and the component fractions of acetone and acetoacetic acid ($r = 0.7037$), and β-hydroxybutyric acid ($r = 0.9202$).

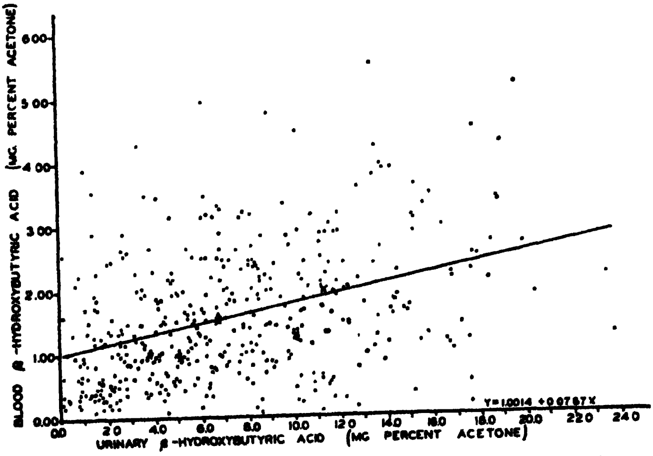

FIG. 8. Comparison of blood and urinary β-hydroxybutyric acid. (× = two separate values falling on the same point.)

CONCLUSIONS

The data presented in this paper indicate that factors such as stage of lactation, parturition, gestation, and sudden cessation of milking do not ordinarily exert much influence upon the concentrations of blood and urinary acetone bodies and their component fractions of acetone and acetoacetic acid, and β-hydroxybutyric acid. If any of these factors had any effect upon the blood and urinary acetone bodies during these experiments, they were apparently obviated by large day to day and monthly variations due primarily to feeding conditions.

Acetone bodies were found in significant amounts in all of the milk samples analyzed but were usually lower in milk than in blood.

The mean concentration of total acetone bodies in 427 blood and urine samples obtained from 16 cows over a period of 15 months was 2.66 ± 1.23 and 11.81 ± 6.56 mg. per cent acetone respectively.

There was a highly significant correlation between the blood and urinary concentration of the following substances: total acetone bodies, acetone and acetoacetic acid, and β-hydroxybutyric acid.

The average composition of blood acetone bodies was 59.39 per cent β-hydroxybutyric acid and 40.61 per cent acetone and acetoacetic acid when calculated as acetone. The average composition of the urinary acetone bodies was 61.72 per cent β-hydroxybutyric acid and 38.28 per cent acetone and acetoacetic acid when calculated as acetone.

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STUDIES ON KETOSIS IN DAIRY CATTLE. III. BLOOD AND URINARY ACETONE BODIES AS RELATED TO AGE[1]

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In the studies of the normal variations of blood and urinary acetone bodies in dairy cattle, experiments were conducted to observe some of the variations in the concentrations of these substances in relation to age. A number of investigators (2, 6, 7) have suggested that rumen digestion may play an important part in the production of the acetone bodies. If the rumen is involved in the production of the acetone bodies, it might be expected that some differences would occur in the production of these substances with the changes that take place in the development of the rumen.

It also appeared that a relationship may exist between the concentration of the blood and urinary acetone bodies and the physiological processes related to production and reproduction.

EXPERIMENTAL

The following three age groups were studied: calves at birth, heifer calves from birth to 10 months of age, and heifers from 9 to 26 months of age. The same precautions were observed relative to the methods of obtaining samples and the analyses for acetone bodies as in previous work (4, 5). The method of Barnes and Wick (1) was used in the determination of the acetone bodies.

Blood acetone bodies from birth to 10 months of age. The concentration of total blood acetone bodies and the component fractions acetone and acetoacetic acid, and β-hydroxybutyric acid were determined upon eight calves at birth. Samples were also drawn from their dams at the same time for purposes of comparison. Table 1 reveals that the total blood acetone bodies

TABLE 1

Blood acetone bodies of eight calves and their dams following parturition*

	Calves	Dams	F (calves vs. dams)
Total blood acetone bodies	1.28 ± 0.65†	2.45 ± 0.63	21.92
Blood acetone and acetoacetic acid . ..	0.71 ± 0.54	1.37 ± 1.04	6.10
β-hydroxybutyric acid ..	0.57 ± 0.82	1.06 ± 0.77	4.07

* Expressed as mg. per cent acetone.
† Standard deviation.

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[2] The experimental data in this paper are taken from a thesis presented by C. B. Knodt in partial fulfillment of the requirements for the degree of Master of Science in Dairy Industry, University of Connecticut, Storrs Agricultural Experiment Station.

of these calves at birth were considerably lower than those of their dams and of the normal values for mature cows previously reported (4, 5). An analysis of variance showed that this difference between calf and dam was highly significant. Because of the great variations in the composition of the total acetone bodies, there was not a highly significant difference between the calves and their dams in the concentrations of the two fractions.

There was no apparent correlation between the acetone bodies of the calves and their dams. This indicates a certain degree of independency of the calf in the production or utilization of the acetone bodies.

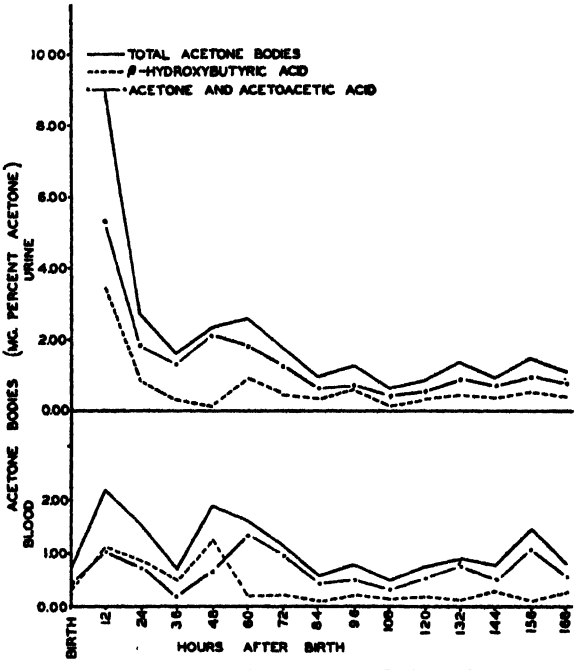

FIG. 1. Blood and urinary acetone bodies of a calf.

Blood and urinary acetone bodies and the component fractions, acetone and acetoacetic acid, and β-hydroxybutyric acid of a Jersey heifer calf were determined every 12 hours from birth to one week of age. The calf was allowed to remain with its dam for three days following birth, after which it was weaned and fed three pounds of whole milk twice each day. Blood and urine samples were taken at 6 : 30 A.M. and 6 : 30 P.M. and milk was fed at 8 : 00 A.M. and 4 : 00 P.M.

As indicated in figure 1, the blood and urinary acetone bodies were extremely low during the first seven days following birth. During this period most of the blood and urinary acetone bodies consisted of the acetone and acetoacetic acid fraction. During the first three days following birth the values for blood and urinary acetone bodies were higher than the following four days. Although the morning samples were taken one and one-half hours before feeding and the evening samples two and one-half hours after feeding, there was no essential difference in the level of blood and urinary acetone bodies. This is of considerable interest since previous work (4) has shown a marked rise in blood acetone in mature cows following feeding.

Blood acetone bodies were determined at short intervals on four calves from birth to 10 months of age, and urinary acetone bodies were determined on these calves from the third day after birth to 10 months of age. The results are shown graphically in figure 2. The blood acetone bodies were quite low for the first two months, after which there was a progressive increase extending over the entire period. The urinary acetone bodies were extremely low the first two weeks, being less than 2.0 mg. per cent. From the second week to the second month after birth the urinary acetone bodies increased about 400 per cent, and continued to increase with some fluctuations during the remainder of the period. It will be noted that the blood and urinary acetone bodies during the first few weeks were composed primarily of acetone and acetoacetic acid. After the third month the urinary β-hydroxybutyric acid fluctuated widely, whereas the acetone and acetoacetic acid remained more nearly constant. The increase in total urinary

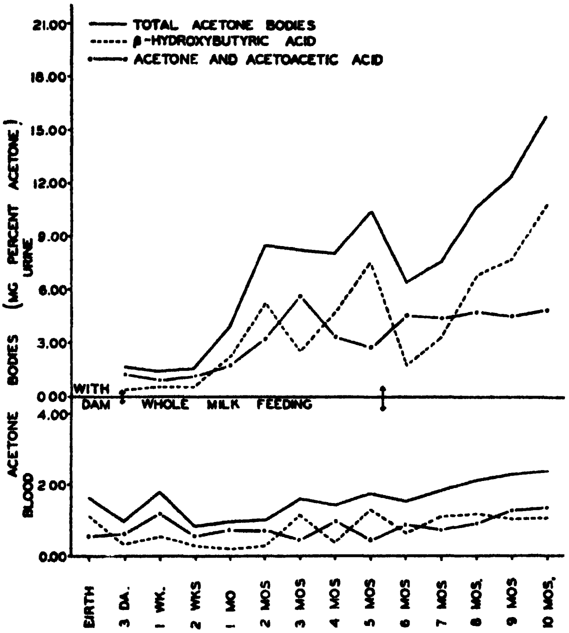

Fig. 2. Blood and urinary acetone bodies of calves from birth to ten months of age. (Average of 4 calves.)

acetone bodies from the seventh to the tenth month was due entirely to β-hydroxybutyric acid.

Blood and urinary acetone bodies from 9 to 26 months of age. Blood and urinary acetone bodies of five heifers were studied over a 12-month period. At the beginning of the experiment the heifers ranged from nine to 13 months of age. Three of the heifers were pasture fed during the summer and received hay and grain during the winter period. Two of the heifers were fed identically with the exception that they received no grain during the winter.

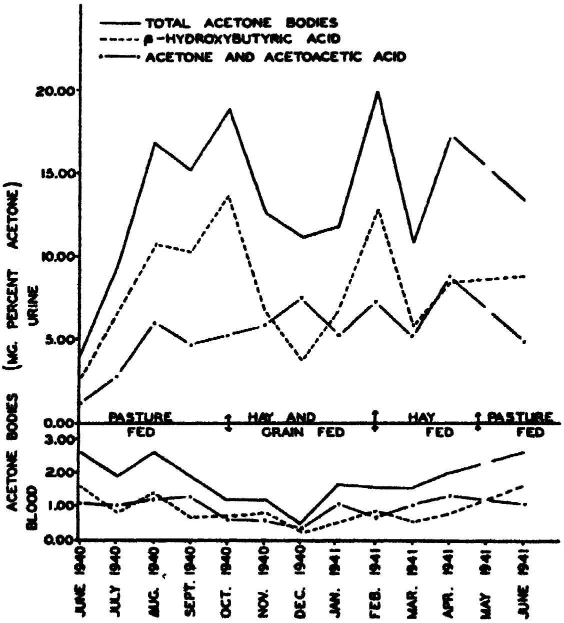

FIG. 3. Blood and urinary acetone bodies of 3 heifers 12 to 13 months of age as of June 1, 1940.

Since the feeding of grain appeared to have some effect upon the acetone bodies, the heifers were divided into two groups. As shown in figures 3 and 4, the blood acetone bodies of the three heifers receiving hay and grain during the winter period were markedly lower than those of the two heifers which received only hay during this period. As in the case of the older heifers on the same pasture (4), the blood acetone bodies were more concentrated while the heifers were on pasture than at any other period. This appeared to be in contradiction with previous experiments upon mature cows (4) in which it was observed that there was a decrease in the blood acetone bodies associated with pasture feeding.

The total urinary acetone bodies were extremely variable in these two groups of heifers and most of the variations in the total urinary acetone bodies were due to β-hydroxybutyric acid.

Comparison of blood and urinary acetone bodies of various age groups of dairy cattle. For purposes of comparison the data presented in table 2 show the relationship of age to the concentration of acetone bodies in blood and urine. Apparently the blood and urinary acetone bodies of the two youngest groups were considerably lower than for the older heifers and mature cows. The higher concentration of blood acetone bodies in the mature group as compared to the group 9 to 26 months of age may have been due to the effect of silage feeding to the former group. It would be expected that the urinary acetone bodies of the mature group would also be higher. This, however, was not true, as the urinary acetone bodies of the younger group were somewhat higher than those of the mature cows. The reason for this is not apparent.

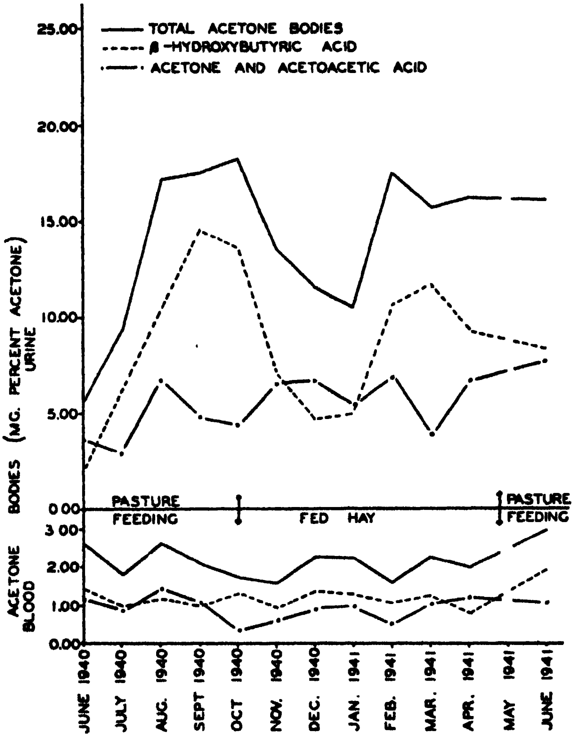

FIG. 4. Blood and urinary acetone bodies of 2 heifers 9 months of age as of June 1, 1940.

The gradual increase in the acetone bodies of the calf during the first few months after birth appears to parallel roughly the development of the

TABLE 2

Comparison of blood and urinary acetone bodies of various age groups of dairy cattle*

Age group (months)	No. of analyses	Mean	S.D.	Min.	Max.
Total Blood Acetone Bodies					
Birth	8	1.28	0.75	0.31	2.43
Birth to 10	65	1.49	0.66	0.49	3.86
9–26	100	2.04	0.73	0.43	3.81
Mature	427	2.66	1.23	0.31	6.95
Blood Acetone and Acetoacetic Acid					
Birth	8	0.71	0.17	0.20	1.55
Birth to 10	65	0.81	0.11	0.17	1.46
9–26	100	0.98	0.45	0.10	2.29
Mature	427	1.08	0.66	0.12	5.62
Blood β-hydroxybutyric Acid					
Birth	8	0.57	0.84	0.01	2.23
Birth to 10	65	0.68	0.59	0.07	3.34
9–26	100	1.06	0.60	0.17	2.95
Mature	427	1.58	1.00	0.06	5.52
Total Urinary Acetone Bodies					
Birth	1	1.17	.	..	
Birth to 10	65	6.42	4.88	0.60	20.24
9–26	100	14.30	5.67	3.60	26.26
Mature	427	11.81	6.56	0.61	34.26
Urinary Acetone and Acetoacetic Acid					
Birth	1	0.93	.	.....	.
Birth to 10	65	2.94	1.97	0.20	5.40
9–26	100	5.31	2.48	0.34	11.44
Mature	427	4.52	2.85	0.04	19.78
Urinary β-hydroxybutyric Acid					
Birth	1	0.24	..	.	..
Birth to 10	65	3.48	3.56	0.03	16.12
9–26	100	8.99	4.55	1.28	20.02
Mature	427	7.29	4.78	0.04	23.82

* Expressed as mg. per cent acetone.

rumen and indicates that the rumen may be responsible for the relatively large concentration of acetone bodies in the blood and urine of the bovine. However, during this period there is a gradual decrease in blood glucose (3) which may be associated with the increase in the level of blood and urinary acetone bodies. The high glucose level following birth may in turn be closely associated with the high liver glycogen content at this time (8), so that the apparent relationship between blood glucose and blood acetone bodies may be due to some extent to a more direct relationship between the liver glycogen and the blood acetone bodies.

CONCLUSIONS

Blood acetone bodies of calves from birth to two months of age were lower than at any other time. Urinary acetone bodies were extremely low for the first two weeks following birth after which they increased at a rapid rate.

Blood acetone bodies of the young calves were predominately acetone and acetoacetic acid, whereas β-hydroxybutyric acid usually made up the larger percentage in the older animals.

Heifers receiving pasture grass alone exhibited higher concentrations of blood acetone bodies than when receiving hay and grain. When hay alone was fed the blood acetone bodies were somewhat higher than when hay and grain were fed and somewhat lower than on pasture grass alone.

The concentration of acetone and acetoacetic acid in the urine was less variable than β-hydroxybutyric acid, the latter being largely responsible for the fluctuations in the total urinary acetone bodies.

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INFLUENCE OF pH, TYPE OF FAT, AND PANCREATIC EXTRACT UPON LIPOLYSIS IN HOMOGENIZED MILK*

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In a previous paper (2) results were presented on studies of lipolysis in homogenized milk and cream, which indicated that lipase activity in homogenized milk was not always affected to the same extent, if at all, by those factors which influence the enzymic action in unprocessed raw milk. The results presented herein constitute a continuation of these studies.

The influence of pH on lipase action in homogenized milk has been studied only to a limited extent although Dorner and Widmer (1) observed that acidifying homogenized raw milk with HCl retarded or even completely prevented rancidity development. However, in studies on non-homogenized milk, Mattick and Kay (4) found fat hydrolysis to be greatest at pH 8.2–8.7 and Roahen and Sommer (7) secured a somewhat similar optimum range of pH 8.4–8.6.

The non-specificity of milk lipase has been indicated by many workers in that certain specific fat acid esters have been utilized as the substrate for lipase action. Rogers, Berg and Davis (8) early used ethyl butyrate as a base for lipase action and more recently Mattick and Kay developed a method for measuring activity of the fat splitting enzyme in milk by its ability to hydrolyze tributyrin. Tarassuk and Palmer (9) indicate that milk lipase will hydrolyze the esters of lauric, myristic and palmitic acids. Later, Palmer and Hankinson (6) dispersed diglycol laurate, diglycol oleate, ethyl myristate and butyl stearate in raw skim milk by shaking, and secured hydrolysis in every case.

Hydrolysis of butterfat may be produced by enzymes from other sources than milk. Palmer (5) used a glycerine extract of pigs pancreas and later commercial steapsin to produce lipolysis. Tarassuk and Richardson (10) produced lipolysis in pasteurized cream with steapsin.

EXPERIMENTAL PROCEDURE

Conditions of processing were, in general, identical with those reported previously (2). Adjustments of pH were made with solutions of HCl and NaOH and determinations were by a Beckman pH meter and an E-type glass electrode.

Titrations for free fatty acids were conducted as before with the exception that a 10-gram sample and 0.05 N NaOH were used. One-half the

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values secured in this determination represent the acid degrees of the fat, *i.e.*, the milliliters of N NaOH per 100 grams fat.

In all cases where the acidity of the fat was determined, homogenization was at 500–700 pounds. In other trials, where acidity changes in the fat-skim milk emulsion were measured by titration, homogenization was at 2,500 pounds.

EXPERIMENTAL RESULTS

Influence of pH. Two series of trials were conducted with the view of ascertaining the influence of pH upon lipolysis. In the first series, purified butter oil was homogenized into rennet whey at 500–700 pounds pressure to secure a product containing approximately five per cent fat. Following homogenization, the mixture was divided into five lots which were treated as follows: Lot 1—control, pH approximately 6.5; Lot 2—adjusted to pH 4; Lot 3—adjusted to pH 5; Lot 4—adjusted to pH 8; Lot 5—adjusted to pH 9. After adjusting the samples to the desired pH, a portion of each was immediately pasteurized and fat secured for titration. The remainder was stored for 72 hours at 35–40° F. and the fat then analyzed to determine the extent of lipolysis.

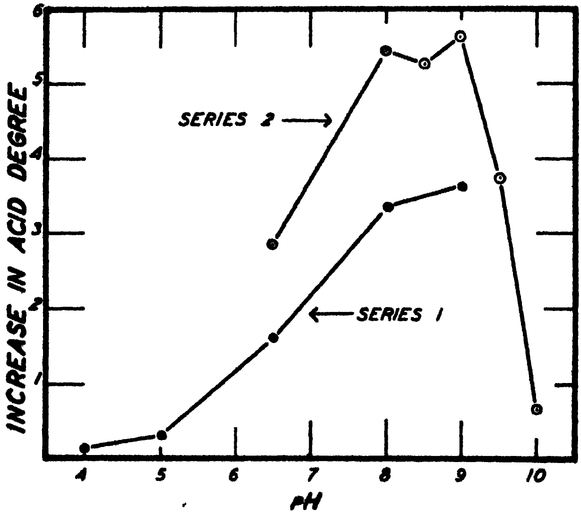

FIG. 1. Influence of pH upon lipolysis in a rennet whey-butter fat mixture.

In the second series, the same conditions prevailed with the exception that the pH was adjusted to 8, 8.5, 9.0, 9.5, and 10.

The results secured are shown by figure 1, in which the values are expressed on the basis of increases in acid degree over the 72-hour period. The maximum rate of lipolysis is within the range of pH 8 to pH 9. In Series 1, the results show no appreciable lipolysis at pH 4 and pH 5, and the results in Series 2 indicates great retardation of fat hydrolysis at pH 10.

The fact that lipolysis is practically prevented when the reaction is at pH 4 or pH 5 raises the question as to the permanency of this inhibition. To study this particular question, an experiment was conducted in which the pH of the fat-whey emulsion was adjusted to near the point of optimum lipase activity, *i.e.,* pH 8–8.5, after it had been at first subjected to pH 4. Then a determination was made to ascertain if any appreciable lipolytic activity persisted.

In the first series of trials in this connection, the casein was precipitated from raw skim milk with HCl by adjusting the reaction to approximately pH 4. The acid whey was then secured by filtration and was homogenized with butter oil to give a product containing about five per cent fat. This emulsion was then divided as follows into two lots: Lot 1—Acidity as secured from acid precipitation of casein, reaction approximately pH 4; Lot 2—Reaction adjusted to pH 8.5 with NaOH. Fat from a portion of each lot was analyzed at 0 and 72 hours. At the end of the 72-hour period, a portion of the lot held at pH 4 was then adjusted to pH 8.5 and held an additional 72 hours. Fat samples were secured immediately after making the change in reaction and also after 72 hours. The results of three trials are presented in table 1.

TABLE 1

The influence of low pH and subsequent readjustment to high pH upon the lipolytic activity of acid and rennet whey

Series 1—acid whey	Trial 1	Trial 2	Trial 3
	acid degree	*acid degree*	*acid degree*
(1) pH 4–4.4,* 0 hours	0.70	0.55	0.75
(2) pH 4–4.4, 72 hours	0.70	0.60	0.90
(3) pH 8.5, 0 hours	0.45	0.25	0.45
(4) pH 8.5, 72 hours	0.50	0.25	0.75
(5) Sample (2), adjusted to pH 8.5, 0 hours	0.50	0.75	0.55
(6) Sample (5) after 72 hours	0.40	0.30	1.30

Series 2—rennet whey	Trial 1†		Trial 2†	
	0 hours	72 hours	0 hours	72 hours
	acid degree	*acid degree*	*acid degree*	*acid degree*
Control—normal pH	1.49	2.56	1.35	3.88
Acid exposure—0 time	0.55	0.64	0.60	1.84
Acid exposure—1 hour	0.55	0.73	0.43	0.72
Acid exposure—18 hours	0.67	0.68	0.44	0.57

* Trials 1 and 2 were adjusted to pH 4, Trial 3 to pH 4.4.
† Trial 1 was adjusted to pH 3.7; Trial 2 to pH 4.13.

In the second series of trials, rennet whey was utilized instead of whey secured from acid precipitation of the casein. In this experiment, the whey was homogenized with butter oil to give a product containing approximately five per cent fat. Immediately after homogenization, a control sample was secured. Fat from this sample was titrated for free fatty acids at once and again after 72 hours of storage. The remainder of the mixture was acidified to about pH 4 (pH 3.7 in one trial, pH 4.13 in another), and then divided into three lots. One lot was immediately adjusted to pH 8; another was held for one hour in the acidified medium and then adjusted to pH 8; the third was held about 18 hours in the acidified condition before it was adjusted to pH 8. In each case, one sample was secured for fat analysis immediately after adjusting to the alkaline range, and another after the mixture had been maintained for 72 hours in the alkaline condition. The results of this experiment are also presented in table 1.

These results, in general, indicate that acid treatment adversely and permanently affects the lipolytic activity of whey, at least under the conditions of this experiment. The acidified samples failed to respond to a marked degree with the alkali treatments, even though exposed to the acid treatment for only a few minutes. However, in those trials in which the reaction was maintained at a somewhat higher pH (Trial 3—Series 1, Trial 2—Series 2), the sample exhibited slight hydrolytic ability.

Type of fat. In another series of trials, a large number of different fats and oils were homogenized into raw and pasteurized skim milk to determine (a) the ability of milk lipase to produce fat hydrolysis in other than milk fat, and (b) the type of flavor produced. The results of two typical trials are presented in table 2.

TABLE 2

Lipolysis in various types of oils and fats when homogenized in raw skim milk

Type of sample	Increase in milliliters of 0.05 N NaOH for 18 gram sample during 96 hours			Flavor
	Trial 1	Trial 2	Avg.	
Control (skim milk)	0.10	−0.10	0.0	Normal
Cottonseed oil (2%)	1.60	1.10	1.35	
Cottonseed oil (4%)	1.80	1.30	1.55	Bitter, oily
Cottonseed oil (6%)	2.50	1.70	2.10	
Control (skim milk)	0.05	−0.10	−0.03	Normal
Castor oil (4%)	1.90	1.90	1.90	Bitter, oily
Coconut oil (4%)	3.00	1.90	2.45	Bitter, soapy, rancid-like
Corn oil (4%)	1.10	1.40	1.25	Bitter
Linseed oil (4%)	1.05	1.00	1.03	Bitter, oily
Olive oil (4%)	1.35	1.20	1.28	Bitter, oily
Control (skim milk)	0.0	−0.05	+0.03	Normal
*Margarine (4%)	1.3	1.00	1.15	Slight bitter
*Hydrogenated cooking fat (4%)	1.6	1.15	1.38	Slight bitter
*Lard (4%)	1.2	1.10	1.15	Slight bitter

* Melted at 100° F. before emulsifying in skim milk.

The results show appreciable fat scission to have occurred in each case when the fat was homogenized in raw skim milk whereas no such change was indicated in the pasteurized sample. Increases in the titer of 18-gram raw samples containing fat varied from 1.0 to 3.0 ml. of NaOH for the different oils and fats studied.

All raw samples, with the exception of the control, developed a bitter flavor upon storage. The intensity of this flavor varied to some extent between different samples, with the oils producing a greater intensity of flavor than the solid fats. Because of the absence of the lower fatty acids in the substrates with the exception of coconut oil, the flavor which developed was not identical with the so-called rancid flavor of milk. However, the coconut oil sample gave a somewhat similar flavor.

Pancreatic extract. It is well known that milk fat may be hydrolyzed by lipolytic enzymes from various sources. The role homogenization may play in such action, however, has not been established. In this connection, trials were conducted in which unhomogenized and homogenized milk were treated with a commercial pancreatic extract. The pancreatic extract was one which has been recommended to be used for producing soft curd milk and as a preventive of oxidized flavor.

The pancreatic extract was placed in a water solution and then added at the rate of four grams to 100 pounds of milk. In one series of trials, the extract was added to raw milk before homogenization, in another, the extract was added to pasteurized milk following homogenization. In every trial, the milk was exposed to the action of the pancreatic extract for exactly 10 minutes after which it was either immediately homogenized and pasteurized or merely pasteurized, depending upon the experiment. Fat was secured from the samples and titrated for free fatty acids. The results are presented in table 3.

TABLE 3

Influence of pancreatic extract on lipolysis in unhomogenized and homogenized milk

Series No.	Treatment	Acid degree
1 (Raw milk)	Unhomogenized	0.60
	Pancreatic extract and unhomogenized*	1.06
	Homogenized†	1.65
	Pancreatic extract then homogenized*	11.06
2 (Raw milk)	Unhomogenized	0.83
	Homogenized†	1.58
	Homogenized*	2.95
	Homogenized—pancreatic extract*	10.54
3 (Pasteurized milk)	Unhomogenized	0.69
	Unhomogenized—pancreatic extract*	1.25
	Homogenized†	0.74
	Homogenized—pancreatic extract*	10.44

* Held 10 minutes before pasteurizing.
† Pasteurized immediately after homogenization.

As expected, the pancreatic extract produced lipolysis whenever it was used. However, the degree of lipolysis produced in the homogenized milk was phenomenal. Whether the extract was added prior to or subsequent to homogenization made no distinguishable difference in the rate of its hydrolytic action. Neither did previous pasteurization of the milk affect the results, indicating that physical changes created by homogenization were the responsible factors. Acid degrees of the fat from homogenized milk to which pancreatic extract was added averaged about 10.7, whereas in the unhomogenized milk under the same conditions, the average acid degree was about 1.2. Naturally, homogenization of raw milk increased the free fatty acids, but this increase was relatively small in comparison to results secured when both homogenization and extract were used.

Rancidity was also produced in homogenized milk treated with pancreatic extract. Homogenization pressures of 500 and 2,500 pounds were both sufficient to produce rancid flavored milk when the milk was exposed to pancreatic extract action for 10 minutes. Furthermore, this flavor change occurred both in raw and pasteurized milk.

DISCUSSION

The fact that the optimum pH for lipase action in the homogenized whey-fat mixture was within the range of pH 8 to pH 9 indicates practically identical conditions in this respect between lipolysis as accelerated by homogenization and lipolysis in unprocessed or normal raw milk. This range agrees with the optimum values of pH 8.2–8.7 of Mattick and Kay (4) and pH 8.4–8.6 of Roahen and Sommer (7) for normal raw milk lipase. If there are several lipases in milk, as has been suggested (1, 3) and if the one which plays the principal role in homogenized milk is different from that which is the most prominent in the lipolytic activity of normal raw milk, then it does not appear logical to attempt to separate them on the basis of different optimum pH requirements.

The failure of acidified whey to exhibit appreciable lipolytic ability even though the pH was readjusted to the optimum pH range, indicates that the acidification permanently affects the fat hydrolyzing enzyme in milk. This is an important consideration if one attempts to concentrate or isolate milk lipase. On the basis of the results in this study, it would appear that any such attempt would require the reaction to be maintained in or near the alkaline range if appreciable enzymic activity is to be retained.

The studies with the various fats and oils indicate milk lipase possesses ability to hydrolyze a large number of different fatty substrates. The fats used varied widely in chemical composition and physical characteristics. However, all possess a certain proportion of unsaturated fatty acids which are likely responsible for at least an appreciable portion of the free fatty acids.

One point of interest in connection with these various fats is that in every case a bitter flavor was produced on lipolysis. Doubtless, a similar type of bitter flavor is produced in milk on the hydrolysis of butterfat but is often overshadowed by the more typical rancid flavor produced by the lower fatty acids. Bitterness is frequently observed in so-called "rancid" milk, the bitterness apparently varying to a large extent between samples, perhaps because of the degree of lipolysis which has occurred. It is possible, therefore, that the rancid flavor of milk is due to a combination of two types of flavors: a putrid type caused by the freeing of butyric and perhaps other acids which will produce putridity and, secondly, a bitter type caused by freeing of certain other fatty acids capable of producing bitterness. The increase in titer of the fat is probably closely allied to the bitter producing fatty acids.

The interesting point relative to the studies with the pancreatic extract is the tremendous rapidity with which lipolysis is produced in the homogenized milk. When one considers that only a 10-minute exposure time is involved, then the extent of fat splitting becomes all the more remarkable. The question remains as to whether the action of the extract is favored by the smallness of the fat globules as produced by homogenization or by the change in the adsorbed layer around the newly created fat surfaces.

CONCLUSIONS

Optimum pH for lipase action in a homogenized rennet whey-fat emulsion was within the range of pH 8 to pH 9. Low pH values adversely and permanently affect lipase activity.

Milk lipase is a non-specific fat splitting enzyme capable of producing lipolysis upon a wide variety of fatty substrates under favorable conditions.

Homogenization creates a condition which greatly enhances lipolysis as produced by pancreatic extract.

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A COMPARISON OF RATS FED AN EVAPORATED MILK WITH THOSE FED A "MILK" IN WHICH THE NATURALLY OCCURRING FAT HAS BEEN REPLACED BY COCONUT OIL

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Milk substitutes are used in human nutrition and it is important to know their effect upon the body. One form of substitute for milk results from the addition of some other fat to skimmed milk. Several reports have recently appeared in the literature comparing the nutritive value of butterfat with that of various vegetable oils. Schantz, Elvehjem and Hart (15) showed an increased rate of growth in rats receiving butterfat as compared to those receiving corn, coconut, cottonseed or soybean oil homogenized in skimmed milk. Gullickson and Fountaine (8) reported results on calves which indicated superior nutritive properties for butterfat as compared to certain vegetable oils. Harris and Mosher (9) have reported a greater growth of coconut oil-fed adolescent rats than of those fed a similar diet containing butterfat, while Harris and Rosenfeld (10) reported a better growth of weanling rats fed butterfat than in those receiving coconut oil.

The present study is a comparison of the growth, bone ash, liver and tissue fat of rats fed an evaporated milk with animals receiving a "milk" in which the butterfat had been replaced by coconut oil. Both products were procured on the open market and, according to the claims of the manufacturer, contained similar amounts of vitamins A and D.

EXPERIMENTAL METHODS

Animals. All animals were of the Wistar strain and from the same colony. The sexes and litters were divided equally into two groups. All animals were caged separately and weighed every three days.

Analytical procedures. Liver fat was determined on the fresh organ by the method of Best, Channon and Ridout (1). The total and volatile fatty acids of the storage fat were determined on the alcohol-ether (3:1) extract of pooled peri-renal and mesenteric fat. The total fatty acids of the storage fat were estimated by titration, using a modification of the procedure described by Stoddard and Drury (16). On another aliquot of the alcohol-ether extract, the steam distillable fatty acids were determined, using the stills and technique described by Friedemann (5), but with the following adaptations: An aliquot of the alcohol-ether extract was transferred to the steam distilling flask and made up to a volume of 20 cc. with ethyl

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alcohol. One-tenth cc. of saturated sodium hydroxide was added and the extract evaporated to dryness on the steam bath. Fifteen cc. of distilled water was then added to the saponified residue and after the soaps had gone into solution the reaction was made acid to Congo red by the addition of M/1 phosphoric acid. The procedure from then on was as described by Friedemann except that the distillate of the second distillation was collected in an equal volume of 95 per cent ethyl alcohol. The final distillate was brought to a boil and titrated with freshly diluted N/100 sodium hydroxide, using phenolphthalein as an indicator. Generally, the distillate was collected in 100-cc. portions. Blanks were run on reagents and on unsaponified aliquots of the alcohol-ether extract. From these data the percentage of fatty acid acidity due to volatile fatty acids was calculated.

The percentage of bone ash was determined by ashing to constant weight at 500° C. after defatting the bones by continuous extraction with hot alcohol for 24 hours, followed by a similar extraction with ethyl ether.

Pooled samples of 3 cans of evaporated and "filled" milk were selected at random and analyzed by standard procedures (11) for fat, nitrogen, total solids and ash. On analysis the evaporated milk was found to contain 26.4 per cent solids, 7.74 per cent fat, 1.06 per cent nitrogen, and 1.70 per cent ash. Analysis of the "filled milk" gave the following results: total solids, 25.4 per cent, fat, 6 per cent., nitrogen, 1.32 per cent, and ash, 1.59 per cent. The evaporated milk was said to contain 325 U.S.P. units of vitamin D per can, and approximately 2000–3000 units of vitamin A, while the filled milk was said to contain 400 units of D and 2000 units of vitamin A.

EXPERIMENT AND RESULTS

The experiment was started when the rats were 25 to 28 days of age. Forty-three animals were fed evaporated milk and 42 animals received the product containing coconut oil. Approximately two-thirds the rats of each group were killed after 49 days on the experiment, and the remainder at 97 days. The two products were each fed to the respective groups of rats *ad libitum.* In addition, 0.25 mg. of iron, 0.05 mg. of copper and of manganese were fed separately as a supplement daily in a small amount of each food. The major portion of food was withheld until the supplement had been ingested.

The data obtained on these animals are summarized in table 1 and their growth curves are shown in figure 1. The growth curves for the animals carried for 97 days on the experiment are also included in the curves of the animals sacrified after 49 days. Growth on both evaporated and "filled" milk continued throughout the experiment. Growth was somewhat greater on the evaporated milk diet for both the 49- and 97-day animals. The difference in growth on the two diets is not statistically significant after 49 days, but is significant after 97 days (7). The percentage of bone ash is almost identical on the two diets, and the amount of liver fat is also similar. There is a definite difference in the amount of volatile fatty acids of the depot fat. The average value for the group given evaporated milk was 5.0 per cent of the total fatty acid acidity after 49 days on the diet, while the "filled milk" group gave a volatile fatty acid acidity equal to 16.3 per cent of the total storage fatty acid acidity. Hemoglobin determinations made on 10 rats from each group after 49 days on the experiment yielded similar results for each group.

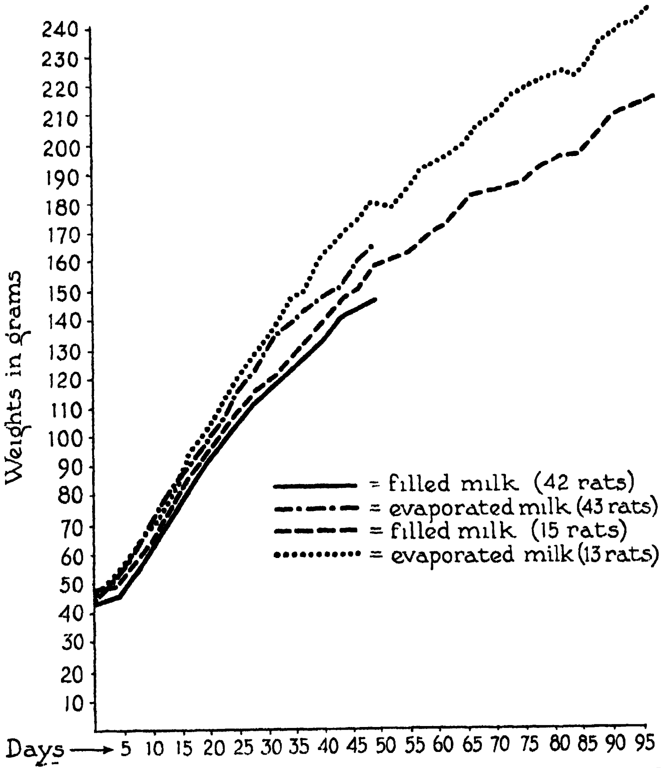

FIG. 1. Growth curves of rats fed evaporated or "filled milk" and a daily supplement of iron, copper and manganese.

During the last 48 days of the 97-day experiment, there was a greater incidence of loose stools passed by the "filled milk" rats than by the corresponding group fed evaporated milk.

Comment. Differences in growth observed in the present experiment cannot be ascribed to any particular aspect of the diet in as much as the diets differed in a number of respects. However, the most striking difference in the diet of the two groups of animals was in the character of the fat. The results of Schantz, Boutwell, Elvehjem and Hart (14) indicate that the

TABLE 1

Growth, percentage bone ash and liver fat of rats fed evaporated milk or a similar product containing coconut oil ("filled milk")

Milk	No. of animals on exp.	Days on diet	Average weights in gms.		Growth	Critical ratio* of growth differences
			Initial	Final		
Evaporated	43	49	43	160	116.9 ± 4.6†	1.64
Filled	42	49	43	149	106.7 ± 4.2	
Evaporated	13	97	46	247	201.0 ± 10.4	2.28
Filled	15	97	45	215	169.5 ± 9.0	
			% Bone ash	% Liver fat	Critical ratio	
					Bone ash	Liver fat
Evaporated	27	49	64.2	6.75	0.04	0.50
Filled	27	49	64.3	6.52		
Evaporated	13	97	67.4	.	1.87	
Filled	15	97	66.6			

* C.R. = $\frac{M_1 - M_2}{\sqrt{\sigma M_1^2 + \sigma M_2^2}}$. Where M_1 and M_2 are the mean values. σ = standard deviation of the mean.

† Standard deviation of mean in gms.

superior nutritive value of butterfat over corn oil resides in the saturated fraction of the fatty acids. The increased incidence of loose stools on the "filled milk" fed group carried for 97 days suggests that part of the difference in growth during the latter half of the experiment may have been due to a difference in gastro-intestinal tolerance for the two diets.

The effects of dietary fat upon the characteristics of the storage fat has been studied by many investigators. Gibbs and Agcaoili (6) showed that the physical and chemical constants of pig lard were altered by feeding the hogs on copra cake, which contains 7 to 15 per cent coconut oil. Apparently, fatty acid chains shorter than 10 carbon are not deposited in the tissues to a significant degree. Davis reported (3) some storage by chickens when tributyrin was injected, but none when it was ingested. Eckstein found no storage of butyric or caproic acids when they were fed to rats (14). Powell (13) recovered only traces of fed caprylic acid in the storage fat, while lauric acid was found up to 25 per cent of the fatty acids of the storage fat. When tricaprin was the sole fat in the diet of white rats, it was found that the storage fat contained 15 per cent capric acid (12). Coconut oil contains a high percentage of short-chained fatty acids (2) and is particularly rich in lauric acid. The steam distillable fatty acids from the rats fed coconut oil was largely contained in the first 100 cc. of distillate. These fatty acids were water-insoluble and were solid at 15 degrees centigrade, so it seems reasonable to assume that they were lauric and capric acid. The volatile fatty acids of the butter fat fed animals were relatively low, and their identity is not apparent.

SUMMARY

The growth of rats fed an evaporated milk was greater over a 97-day period than for a similar group of rats maintained on a "filled milk" in which the butterfat had been replaced by coconut oil. The percentage of bone ash and of liver fat is quite similar for the two groups of rats, both after 49 and 97 days on the diets. There were more volatile fatty acids deposited in the storage fat of the coconut oil group.

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BACTERIOLOGICAL STUDY OF CHOCOLATE MILK*

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INTRODUCTION

The widespread use of chocolate milk in recent years is so well known that it is not necessary to enlarge upon the subject here. Mueller (12) investigated the bacterial flora of a number of commercially-packed chocolate syrups and cocoa powders, and reported that some of them contained large numbers of bacteria. It is obvious that the addition of a non-sterile substance to milk will mean the contamination of the milk. This would be particularly true of the syrups because chocolate milk made from them is not always subsequently heated; whereas, when a cocoa powder is used, the mixture is pasteurized to bring the powder into suspension.

It was the purpose of this study to investigate the effect of adding cocoa powders and chocolate syrups to milk on the bacterial content and keeping quality of the milk.

EXPERIMENTAL

I. *Bacteriological examination of cocoa powders and chocolate syrups*

The first experiment was a preliminary determination of the numbers and types of bacteria present in three brands of cocoa powders and three of chocolate syrups, preparatory to their being used to make chocolate milks. The powders and syrups were all commercially-packed products. Samples A and I of the cocoa powders were American-processed powders, and sample D was Dutch-processed. Of the syrups, samples B and T were made from cocoa powders, and sample S was a syrup flavored with an extract of cocoa. All of the containers were kept sealed and otherwise handled aseptically in order that the samples could be used for the subsequent experiments.

Bacterial counts were made on standard nutrient agar, and tomato agar (Kulp (7)) was employed for detecting the presence of yeasts and molds. Appropriate dilutions, determined by trials, were made in sterile distilled water, and 1-ml. portions of these dilutions were plated. All plates were made in duplicate. One series of duplicate plates was incubated at 37° C., and a parallel series of duplicate plates at room temperature (about 22° C. in the room used). The latter series was intended to estimate the numbers of bacteria present that would develop if the milk made from the products was left standing at room temperature. Bacterial counts were made at the end of 48 hours' incubation. Tomato-agar plates were incubated and examined up to 5 days if no mold or yeast growth appeared before that time.

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Streak cultures, from the suspensions used for plating, were made on blood-agar to detect the presence of hemolytic cocci, and on Endo's agar to detect gram-negative bacteria of intestinal types. These plates were incubated at 37° C., and examined at 24, 48, and 72 hours. Results of the whole experiment are shown in table 1.

TABLE 1

Bacterial counts per gram of cocoa powders and chocolate syrups (plates incubated at 22° and 37° C.)

	Nutrient agar		Endo's agar		Blood agar		Tomato agar*	
	22°	37°	22°	37°	22°	37°	22°	37°
Powder A	10,200	700	–	–	–	–	+	+
" D	80	120	–	–	–	–	+	+
" I	32,000	10,000	–	–	–	–	–	–
Syrup B	49,000	90,000	–	–	–	–	+	+
" S	30	50	–	–	–	–	–	–
" T	220	310	–	–	–	–	–	–

* Tomato agar was used to detect the presence of molds. Since mold growth, when present, covered the whole plate, no attempt was made to secure counts.

The numbers of bacteria in both powders and syrups showed wide variation. This was in agreement with the results published by Mueller (12). It will be noted, however, that no bacteria were recovered on the Endo plates, and no hemolytic organisms appeared on the blood-agar plates. This indicates that neither gram-negative intestinal bacteria nor hemolytic cocci were present in the powders or the syrups. A number of colonies growing on the nutrient-agar plates were examined both microscopically and culturally. Most of them proved to be aerobic spore-forming bacteria of the *Bacillus subtilis* group. Since all of these bacteria would have the same significance in milk—they could impair its keeping quality if they were present in sufficient numbers, but would not endanger the health of consumers—it was not considered necessary to identify them as to species. No gram-negative bacteria were isolated from the nutrient-agar plates, but a few gram-positive cocci were recovered. These were studied sufficiently to identify them as micrococci (Bergey (1)); but since very few of them were recovered, and they were probably of no great significance in milk, they were not identified as to species.

II. *Bacterial counts of chocolate milks made with cocoa powders and with chocolate syrups*

Whether the types of bacteria found in cocoa powders and chocolate syrups are pathogenic or not, substantial numbers of them could affect the keeping quality of chocolate milks. This would be especially true if the chocolate milk were kept at room temperature or at ineffective refrigeration temperature, as might be the case in some homes, or in carelessly operated public eating places.

The powders and syrups examined in the first experiment were used to make chocolate milks. Methods for the making of chocolate milk are not standardized, and the result is considerable variation in cocoa content among the products prepared by different market-milk plants. The milks in which cocoa powders were used were prepared according to the formula employed by the Department of Dairy Industry of the Massachusetts State College.

The powders were added by weight, and the milk was measured on the basis of 1.032 specific gravity. Cane sugar was added to each sample to make the final by-weight composition: 1 per cent cocoa powder, 7 per cent cane sugar, and 92 per cent milk. The ingredients were mixed and heated to 144° F. for 30 minutes to bring the cocoa into suspension. The mix was then cooled rapidly in an ice-water bath.

Chocolate milks were made from syrups by mixing cold pasteurized milk and cold syrup in the proportions recommended by the manufacturers of the respective brands of syrup employed. The mixtures were not heated.

After the chocolate milks had been prepared, each lot was divided into two portions. Plates were made immediately from all portions, using the medium and technic for the bacteriological analysis of milk according to Standard Methods (16). Then one portion of each milk was stored at room temperature, about 72° F. in the room used, and the other portion of each milk was stored in an electric refrigerator at about 43° F., "economy" temperature in the refrigerator employed. These two storage temperatures were employed to represent both satisfactory and unsatisfactory storage conditions. Subsequently, plates were made from all portions after about 7 hours' storage, and again after 24 hours. Plates were made again after 48 hours from the portions in the refrigerator. Two sets of duplicate plates were made each time from each portion, and one set was incubated at 37° C. and the other at room temperature. The use of 37° temperature is the usual practice, and room temperature was employed to obtain counts especially of those organisms that would develop as a result of room-temperature storage.

Circumstances did not permit carrying out the whole series of counts at one time. Consequently, check counts, such as those reported in table 1, were made from the several powders and syrups whenever they were opened to make fresh batches of chocolate milk. Freshly pasteurized batches of milk were used for each of the several preparations of chocolate milk that were necessary. Plate counts of milk were made each time it was obtained.

Results of this experiment are shown in tables 2 and 3. The numbers of bacteria added to milk with the addition of cocoa powders were not very important, except for spores, because the chocolate milk was heated to 144° F. for 30 minutes to bring the powders into suspension. This amounts to pasteurization. Further, only 1 per cent by weight of the milk is cocoa powder;

which would mean that, even when the highest-count cocoa powder was used (see table 1), the bacterial count of the milk would be increased by only a few over 100 per ml. Probably the pasteurization would destroy or inactivate many of these organisms. When syrups were used, however, around 10 per cent by weight of syrup was added to milk, and the mix was not heated. Thus, the bacterial content of the chocolate milk was substantially increased when high-count syrup was used, as can be seen by noting the counts for chocolate milk made with syrup B in tables 2 and 3.

TABLE 2

Bacterial counts of chocolate milks stored at refrigerator temperature (43° F.)

Sample	Plates incubated at 37° C.				Plates incubated at 22° C.			
	Hours of storage				Hours of storage			
	0	7	24	7 days	0	7	24	7 days
Powder A								
Milk	1,150	1,130	1,180	8,100	1,200	1,100	1,400	17,000
Chocolate milk	1,110	1,140	1,180	1,430	1,000	1,100	1,510	3,000
Powder D								
Milk	760	1,220	1,380	7,500	1,100	1,090	1,120	16,400
Chocolate milk	610	800	840	1,300	980	970	970	8,000
Powder I								
Milk	1,600	1,710	1,700	3,300	1,700	3,570	3,200	16,500
Chocolate milk	1,700	1,690	1,640	2,110	1,320	3,400	3,600	5,900
Syrup B								
Milk	1,610	1,580	1,650	4,000	1,660	1,620	2,610	9,000
Chocolate milk	8,400	8,700	8,600	12,300	6,400	6,900	8,400	9,800
Syrup S								
Milk	1,000	1,000	1,600	3,100	1,200	1,000	1,600	2,000
Chocolate milk	800	1,400	2,400	4,000	1,400	1,500	1,550	2,600
Syrup T								
Milk	1,390	1,370	4,100	6,000	2,400	2,300	2,900	13,000
Chocolate milk	1,330	1,400	3,100	3,900	2,200	2,100	2,600	3,100

For all of the milks made from cocoa powders, the counts had not increased much at the 7-hour period, and all of the 24-hour counts were substantially lower than the counts for the corresponding milk samples at room-temperature storage; this was also true for the 7-day counts of samples stored in the refrigerator. This fact indicates that the cocoa powders contained some factor that inhibited bacterial growth in chocolate milks made from them. Counts from chocolate milk made with syrup T showed the same trend as did counts from the milks made with cocoa powder. Syrup B had a high initial count, so the milk made with it had a high initial count compared with the count from the corresponding milk sample, and the count from the chocolate milk remained relatively high throughout. It will be noted, however, that the percentage increases of counts from this chocolate milk were definitely lower than were those from the corresponding milk sample, for both conditions of storage and incubation. These facts indicate that both syrups B and T contained the same growth-inhibiting factors that were present in the cocoa powders. The counts from the chocolate milk made with syrup S followed closely those from the corresponding milk samples. This syrup was flavored with an *extract* of cocoa powder, and had no cocoa in it, so the growth-inhibiting factor was evidently lost in its manufacture.

TABLE 3

Bacterial counts of chocolate milks stored at room temperature (72° F.)

Sample	Plates incubated at 37° C.			Plates incubated at 22° C.		
	Hours of storage			Hours of storage		
	0	7	24	0	7	24
Powder A						
Milk	1,900	4,000	4,500,000	2,400	2,800	20,000,000
Chocolate milk	2,000	3,100	1,700,000	2,400	2,200	8,000,000
Powder D						
Milk	1,300	1,800	6,000,000	1,210	1,750	16,500,000
Chocolate milk	1,800	1,850	2,300,000	860	1,790	3,000,000
Powder I						
Milk	1,220	1,250	4,100,000	1,890	7,500	30,000,000
Chocolate milk	1,300	2,400	2,600,000	1,330	4,600	17,000,000
Syrup B						
Milk	1,760	1,740	1,600,000	1,800	1,890	14,500,000
Chocolate milk	7,800	8,500	1,680,000	9,400	8,400	16,900,000
Syrup S						
Milk	1,290	1,500	2,100,000	1,210	2,800	20,000,000
Chocolate milk	1,300	1,400	1,800,000	1,800	2,200	16,000,000
Syrup T						
Milk	1,340	1,810	3,000,000	1,410	2,100	43,000,000
Chocolate milk	1,000	1,700	1,500,000	1,300	1,700	18,000,000

III. *Growth of certain species of bacteria in chocolate milk*

This experiment was set up to study the growth of certain bacterial species in chocolate milks. The species selected for study were among those that might be found in milk under ordinary conditions, and no specifically pathogenic bacteria were included. The bacterial species were: ***Escherichia coli, Aerobacter aerogenes, Staphylococcus aureus, Streptococcus lactis,*** and ***Bacillus cereus.***

The chocolate milks were made as in experiment II; and the same powders and syrups were used, except that syrup S was omitted because it had not inhibited bacterial growth in the experiment. After the milks had been made up, they were sterilized in the autoclave, and were then inoculated from 24-hour cultures of the respective bacterial species. Duplicate series were stored as before at room temperature and at refrigerator "economy" temperature. Platings were made immediately after the milks had been inoculated, at about 7 hours, and at 24 hours. Platings were made also at 48 hours from the milks stored in the refrigerator. Because of the volume of work involved, and because room-temperature incubation is not standard according to Standard Methods of Milk Analysis, plates were incubated only at 37° C.

Results are shown in table 4. It will be noted that inoculations were

TABLE 4

Growth of pure cultures of bacteria in chocolate milk[*] *(plates incubated at 37° C.)*

	Refrigerator storage				Room storage		
	Hours of storage				Hours of storage		
	0	7	24	48	0	7	24
E. coli							
Milk	980	880	1,190	1,220	1,110	28,100	200,000,000
" + sucrose	1,070	....	1,430	1,520	1,170	18,600	216,000,000
A[*]	920	1,070	1,090	820	930	10,100	85,000,000
D	1,120	1,170	1,180	630	1,200	12,500	93,000,000
I	950	1,110	1,100	560	1,110	15,400	80,000,000
B	990	950	900	650	1,020	6,000	69,000,000
T	900	770	410	290	920	2,200	6,000,000
A. aerogenes							
Milk	1,270	1,300	1,350	1,240	1,360	18,300	297,000,000
" + sucrose	1,310	1,240	1,290	1,180	1,280	14,100	198,000,000
A	1,340	1,290	1,210	1,150	1,310	1,310	39,200,000
D	1,390	.	1,120	1,170	1,270	1,890	63,100,000
I	1,200	1,150	1,270	1,180	1,330	1,980	91,000,000
B	1,350	.	1,230	1,150	1,230	1,570	5,300,000
T	1,250	1,130	1,130	1,110	1,210	1,270	990,000
Staph. aureus							
Milk	6,420	6,370	4,010	2,630	6,390	37,700	35,000,000
" + sucrose	6,610	6,560	3,730	2,150	6,170	35,600	60,000,000
A	5,640	5,710	520	412	5,960	12,300	1,600,000
D	6,120	5,820	1,750	1,670	5,890	21,000	4,900,000
I	6,530	6,180	750	860	5,990	10,700	2,100,000
B	6,370	6,280	1,820	1,220	6,440	9,800	110,000
T	6,100	5,420	970	1,180	6,070	6,300	108,200
Strep. lactis							
Milk	2,200	2,250	2,320	2,710	2,300	84,200	170,000,000
" + sucrose	2,370	2,150	2,080	2,320	2,280	87,600	165,000,000
A	2,120	1,890	1,940	2,130	2,420	75,400	260,000,000
D	2,040	1,720	2,300	2,610	2,010	67,300	149,000,000
I	2,160	1,570	1,790	2,260	2,450	83,200	273,000,000
B	2,060	1,410	1,600	1,670	2,030	37,500	132,000,000
T	2,210	1,480	1,540	1,610	2,460	27,000	126,000,000
B. cereus							
Milk	1,580	1,810	1,950	2,000	1,780	14,800	42,000,000
" + sucrose	1,610	1,590	1,600	2,000	1,800	13,100	50,000,000
A	1,560	1,550	1,800	1,200	1,850	4,510	17,000,000
D	1,650	1,470	1,590	1,400	1,720	4,560	15,000,000
I	1,730	1,580	1,700	1,430	1,810	3,860	22,000,000
B	1,590	1,540	1,680	1,110	1,860	4,420	7,000,000
T	1,600	1,610	1,700	1,000	1,800	3,220	4,000,000

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[*] Letters represent chocolate milks made with cocoa powders A, D, and I, and chocolate syrups B and T.

made in milk containing the amount of sugar (sucrose) used in making the cocoa-powder mixes. This was done to determine the effect of the sugar concentration on bacterial growth. The results of the experiment may be summarized as follows:

Refrigerator storage. No particular effect on the numbers of coliform bacteria was noticeable within 24 hours. At 48 hours the number of *Escherichia coli* were somewhat diminished in all of the chocolate milks, while counts of *Aerobacter aerogenes* were not affected. The counts of *Staphylococcus aureus* decreased, even in the milk, in 24 hours, and they decreased still further in 48 hours. The counts of the organism were lower in the chocolate milks than in the milks without syrup or powder. *Streptococcus lactis* apparently was not much inhibited in any of the milks, although the counts in the milks with syrups B and T were somewhat lower than the counts in the other milks at both 24 and 48 hours. All of the counts of *Bacillus cereus* in chocolate milk, at 48 hours' storage, were lower than those of the milk controls. This result was unexpected in view of the fact that most of the bacteria found in the syrups and powders, in experiment I, were members of the *Bacillus subtilis* group. The explanation is not immediately apparent.

Room storage. Except for *Streptococcus lactis,* the counts of the several bacterial species employed were definitely lower from the chocolate milks than they were from either the control milk or the milk with sugar. In most instances, however, the counts were somewhat increased at 7 hours, and definitely increased at 24 hours. From the milk made with syrup T, counts of all the bacterial species, except *Streptococcus lactis,* were substantially lower than from the other chocolate milks. The same was true of counts of *Aerobacter aerogenes, Staphylococcus aureus,* and *Bacillus cereus* from milk made with syrup B.

In general, it may be stated that, with the exception of some of the counts of *Streptococcus lactis*, the results of the experiment were consistent with those shown in tables 2 and 3, in that they indicated the presence in the chocolate milks of some agent that inhibited bacterial growth. The counts from the milk with added sucrose were about the same as in the milk without it, which indicated that the inhibition of bacterial growth was not attributable to the increased sugar content of the chocolate milks.

IV. *Growth of bacteria in the presence of certain constituents of cocoa and chocolate*

The second and third experiments provided data which suggested that all three of the cocoa powders and two of the chocolate syrups possessed some power to inhibit bacterial growth in chocolate milks made with them. The fourth experiment was set up to attempt to discover the factor or factors responsible for the observed inhibition.

Analyses of cocoa products (Kuzmeski and Mueller (8); Whymper (18)) have shown that tannic substances, theobromine, and oxalic acid are present in sufficient concentrations to suggest that they might be able, singly or in combinations, to affect the rate of bacterial growth in chocolate milk. The concentrations of these compounds vary in different cocoas; but, in general, their approximate percentages seem to be: Tannic substances, 10 per cent; theobromine, 2 per cent; and oxalic acid, 0.65 per cent. In the formula, given in experiment II, for making chocolate milk from cocoa, one per cent by weight of cocoa is added to milk. On that basis the chocolate milk would contain 0.1 per cent of tannic substance, 0.02 per cent of theobromine, and 0.0065 per cent of oxalic acid.

The nature of the tannic substances in cocoa powders, and consequently in chocolate syrups, is not known, but tannic acid is probably not present to any great extent as such. That was the only tannin derivative, however, that was available in pure chemical form for use in the experiment.

Freshly pasteurized milk from the college dairy was used, as before, for the culture medium. The desired quantities of tannic acid, theobromine. and oxalic acid were weighed and added to 100-ml. portions of the milk, and these portions were again pasteurized. The tannic acid, theobromine, and oxalic acid were added, separately and in combination, in amounts that would give approximately the same concentrations as those indicated in the analyses previously mentioned. Sugar (sucrose) was used also with the combined compounds to indicate its effect, if any, on bacterial growth in the presence of the compounds. The same bacterial species were employed as in experiment III, and they were inoculated into the milk and milk-mixes in the same way. Room-temperature storage was employed. Platings were made immediately after the inoculations, and at 7 hours and 24 hours of storage. Plates were incubated at 37° C., and were counted at 48 hours. Results are shown in table 5.

The results obtained indicate that tannic acid alone, and the combination of all three compounds, inhibited the growth of the coliform bacteria, of *Staphylococcus aureus,* and of *Bacillus cereus,* but not of *Streptococcus lactis.* These results agree with the effect observed in experiment III, in which cocoa powders and chocolate syrups were used. Neither oxalic acid nor theobromine alone had any demonstrable effect on bacterial growth. The combination of all three compounds did not appear to have any greater inhibiting property than did the tannic acid alone, except in the combination with added sugar. With the latter combination *Streptococcus lactis, Escherichia coli,* and *Bacillus cereus* all gave lower counts than with tannic acid alone. The general trend of the counts obtained in the experiment indicates that tannic acid was the principal factor in inhibiting bacterial growth. If the action of tannic acid can be accepted as being representative of the action of tannin compounds, these results argue in favor of the tannic substance present in cocoa being the significant factor in the inhibition of bacterial growth observed in chocolate milks.

TABLE

Growth of pure cultures of bacteria in the presence of tannic acid, theobromine and oxalic acid (plates incubated at 37° C.)

Milk, and milk with added substances	Hours of storage at room temperature		
	0	7	24
E. coli			
Milk	3,280	56,300	553,000,000
Sugar, tannic acid, oxalic acid, theobromine	2,940	10,700	76,000,000
Tannic acid, oxalic acid, theobromine	1,910	10,200	291,000,000
Tannic acid	3,000	6,700	261,000,000
Theobromine	3,280	108,000	641,000,000
Oxalic acid	2,960	90,000	582,000,000
A. aerogenes			
Milk	4,040	62,000	574,000,000
Sugar, tannic acid, oxalic acid, theobromine	4,320	27,000	74,000,000
Tannic acid, oxalic acid, theobromine	4,610	21,000	53,000,000
Tannic acid	4,930	26,000	85,000,000
Theobromine	4,870	57,000	392,000,000
Oxalic acid	3,890	64,000	479,000,000
Staph. aureus			
Milk	1,890	17,680	40,300,000
Sugar, tannic acid, oxalic acid, theobromine	1,810	14,870	13,370,000
Tannic acid, oxalic acid, theobromine	1,790	13,960	12,110,000
Tannic acid	1,920	13,280	10,980,000
Theobromine	1,970	18,820	51,400,000
Oxalic acid	1,830	16,240	38,600,000
Strep. lactis			
Milk	1,710		61,000,000
Sugar, tannic acid, oxalic acid, theobromine	1,550	8,900	25,000,000
Tannic acid, oxalic acid, theobromine	1,730	14,900	44,000,000
Tannic acid	1,790	16,900	64,000,000
Theobromine	1,670	13,300	68,000,000
Oxalic acid	1,840	18,800	76,000,000
B. cereus			
Milk	1,780	9,300	7,100,000
Sugar, tannic acid, oxalic acid, theobromine	1,520	8,900	2,600,000
Tannic acid, oxalic acid, theobromine	1,560	7,100	6,200,000
Tannic acid	1,580	11,100	5,300,000
Theobromine	1,630	10,300	7,000,000
Oxalic acid	1,620	17,600	6,400,000

DISCUSSION

The absence of potentially pathogenic bacteria from the cocoa powders and chocolate syrups, examined in this study, agrees with the results reported by Mueller (12). The absence of these types of bacteria is reassuring so far as the safety of chocolate milk is concerned. This is particularly true with respect to gram-negative bacteria of intestinal type, as it indicates that there is little danger of intestinal bacteria, and of typhoid and dysentery organisms particularly, being added to milk when chocolate milk is made. The fact that the growth of bacteria, excepting *Streptococcus lactis,* was inhibited in chocolate milks provides an additional margin of safety. An exception would be a chocolate milk made from an extract-flavored syrup such as syrup S.

Kliewe and Strawe (5) reported that cocoa products have definite germicidal power, and they added that this is the reason why "contamination of cocoa with intestinal bacteria has never been reported." The results of the present experiments do not argue in favor of the powders and syrups having sufficient germicidal power to render an infected milk safe by their addition to it, but there would seem to be little danger that milk would be dangerously contaminated from the addition of cocoa products, or that the bacterial count would be substantially increased in the chocolate milk unless a powder or syrup of unusually high bacterial content was used.

Tannic acid added to milk definitely inhibited bacterial growth in milk. This was true when tannic acid was used alone or in combination with theobromine and oxalic acid. The effect of tannic acid on bacteria has been studied by several investigators. Taylor (17) found that only small concentrations of tannic acid were required to inhibit growth of pyogenic cocci. Ko (6) stated that tannic acid was a more effective germicide for typhoid bacilli than were other fruit or vegetable acids. Olitsky and Cox (14) treated the nasal passages of mice with tannic acid and found them resistant to subsequent nasal infection with equine encephalomyelitis virus; and Sabin, Olitsky, and Cox (15) found that monkeys similarly treated were resistant to subsequent nasal infection with poliomyelitis virus.

As was stated before, the nature of the tannin complex in cocoa products is not known, and there may be some question as to how accurately the use of tannic acid may represent the action of the tannin complex. Perhaps a partial answer to this question may be found in the work of Doelger (2, 3, 4), who reported that bacteria in tan liquors are largely acid-producing organisms, with lactic, acetic, and butyric-acid producers in greatest abundance. *Streptococcus lactis* was the chief producer of lactic acid. Coliform bacteria have been found in weak tan liquors, but chromogenic bacteria have seldom been encountered. These results compare favorably with those of the present study in that the growth of *Streptococcus lactis* was not inhibited in either chocolate milks or in the presence of tannic acid, but the growth of coliform bacteria and *Staphylococcus aureus* was inhibited in both situations. The results of the present experiments suggest that the tannin complex of the cocoa products was the active factor in the inhibition of bacterial growth.

Lipman and Mueller (9) reported that when a ration containing milk powder and cocoa powder was fed to albino rats, the digestibility of the milk protein was reduced in comparison with its digestibility in rats that received no cocoa powder. In a similar study, Mueller and Ritchie (13) found that cocoa powder in the diet retarded the growth of albino rats. Mueller (11) reported that theobromine (0.27 per cent) in a diet was nontoxic for rats; 2 per cent crystalline tannic acid was toxic; and the toxicity of cocoa powders was related to their quantity of tannic substance. Toxicity was measured by growth of the rats. Mehlitz and Maass (10), and Zlatarov and Poppov (19) reported that tannic acid, even in small quantities, had an inhibiting effect on enzymes. While the several studies mentioned are not concerned with bacteria, they do suggest a certain parallel with the results obtained in the present study.

It was interesting to note that the palatability of the chocolate milks employed was parallel to their bacteria counts. All samples of chocolate milk stored in the refrigator were palatable at 48 hours, as was to be expected. All of these samples were kept in the refrigator and tasted again at the end of 7 days. All of the samples made with the cocoa powders, and that made with syrup T, were still palatable. The respective milk samples, and the chocolate milks made with syrups B and S, were slightly rancid. Samples stored at room temperature were tasted at 24 hours, and all were palatable except those made with syrups B and S. These were definitely unpalatable at this time, as were the respective control milk samples. The spoilage of the milks made with the two syrups can be accounted for by the fact that syrup B had a high initial bacterial count, and syrup S was flavored with a cocoa extract. The chocolate milks made with the syrups were not pasteurized after they were mixed as were the milks made with the cocoa powders.

SUMMARY

A bacteriological study was made of chocolate milks containing, respectively, three brands of cocoa powder and three of chocolate syrup.

A preliminary experiment showed that two of the powders and one of the syrups had high bacterial counts, and the others had low counts.

Growth of the bacterial flora of the milk itself was inhibited when powder or syrup was added, except with one syrup (S) which was flavored with an extract of cocoa powder.

Growth of pure cultures of bacteria was inhibited in the chocolate milks, excepting growth of *Streptococcus lactis*. Other species of bacteria employed were *Escherichia coli*, *Aerobacter aerogenes*, *Staphylococcus aureus*, and *Bacillus cereus*. Syrup S was not used in this experiment.

Tannic acid added to milk inhibited the growth of the same bacterial species as those mentioned in the preceding paragraph, and the growth of *Streptococcus lactis* was not hindered.

Parallel groups of samples were stored at room temperature (72° F.) and at refrigerator temperature (43° F.) for the experiments on the milk flora and pure cultures in chocolate milk. For the experiment with tannic acid, oxalic acid, and theobromine, only room-temperature storage was used. The inhibition of bacterial growth at room temperature was more marked because of the greater growth-rate of bacteria at that temperature as compared with refrigerator temperature.

Oxalic acid and theobromine did not have any noticeable effect on the growth of bacteria.

The palatability of the chocolate milks, and especially of those samples

stored at room temperature, was directly comparable with the bacterial counts of the milks.

The conclusion seems to be justified that cocoa powders or chocolate syrups (excepting those flavored with cocoa extracts) added to milk definitely inhibit the growth of bacteria likely to be found in milk, with the exception of *Streptococcus lactis,* and that the tannic substances of the cocoa products are the agents responsible for inhibiting bacterial growth.

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THE EFFECT OF STILBESTROL AND ANTERIOR PITUITARY EXTRACT UPON LACTATION IN GOATS*

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The production of fully developed lactating mammary glands during pregnancy requires a high degree of synchronization between the pituitary, ovaries and placenta. The entire mammary developmental process can be stimulated by the artificial administration of now available natural and synthetic hormones to virgin or castrate animals. The administration of a synthetic estrogen (diethylstilbestrol) which has many physiological properties of the natural estrogens, to animals with intact pituitaries, causes the development of complex mammary duct systems such as are present in the virgin but mature animal (Turner for review, 51). In at least two species, the guinea pig and monkey, estrogens cause the development of complete mammary glands with lobule-alveolar systems such as are present at mid-pregnancy. But in most species so far studied the addition of progesterone, the corpus luteum hormone, to this estrogen treatment greatly facilitates the growth of the mammary gland and carries the development to completion in animals with intact pituitaries (51).

In addition to their mammary growth-stimulating properties, estrogens have the ability to cause a great increase in the secretion by the anterior pituitary of the lactogenic hormone, which is necessary for the initiation and maintenance of lactation (44, 31, 36). This simulates the condition at normal parturition when estrogenic action becomes dominant over that of progesterone and lactogen secretion pours into the blood and causes the great surge of milk secretion at that time (37, 38). Lactation after parturition comes from fully developed lobule-alveolar glands the growth of which has been stimulated by estrogen and progestin during pregnancy. With the administration of estrogen, however, milk secretion can be induced in less fully developed glands.

The synthesis by English workers of a drug, diethylstilbestrol, which has many of the properties of the natural estrogens and in addition is active on oral administration and can be produced cheaply has stimulated experimentation on the stimulation of growth of the mammary gland and lactation in dairy animals.

Folley *et al.* (17) reported that diethylstilbestrol dipropionate ointment applied to the udders of three virgin goats caused copious lactation. Three virgin heifers responded only slightly (17, 18). Lewis and Turner (29, 30)

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induced lactation in two virgin kids, one of which was a castrate, and in two yearling does given subcutaneous injections of diethylstilbestrol. An aged, sterile cow lactated from the single normal quarter of the udder on diethylstilbestrol treatment (28). Walker and Stanley (52) secured a maximum of 14 and 16 pounds of milk daily from a castrate heifer and a sterile cow after several months' injection of diethylstilbestrol dipropionate plus testosterone propionate. Folley and Young (21) showed that a crude anterior pituitary extract would increase the milk yield of a diethylstilbestrol treated goat or bring into heavy lactation one which had not responded to the estrogen.

Lactation has also been reported from goats due to the action of other estrogens plus anterior pituitary extract. DeFremery (11) reported that percutaneous inunction of the udder with estradiol benzoate caused the development of goat udders of parturient type. Anterior pituitary extract then produced abundant lactation. Trautman and Kirchhof (50) gave orally a total extract of ovary (Oestruzyl) plus an anterior pituitary extract and secured from 480 to 900 ml. of milk daily from several goats.

Anterior pituitary extract alone was reported by Evans (13) to cause considerable lactation in virgin and dry goats and cows whereas Catchpole *et al.* (9) and Asdell (2) had little success with this treatment.

PROCEDURE

During subcutaneous administration of diethylstilbestrol, the chemical was given once daily dissolved in oil. Injections were made in front of or behind the shoulders. For percutaneous administration diethylstilbestrol was dissolved in 70 per cent alcohol. Sufficient carrier was used to cover the udder when released at the dorsal margin and allowed to run over the skin with some manual spreading. The long hairs were clipped from the udder at the beginning of the experiment. The alcohol quickly evaporated. For oral administration diethylstilbestrol was mixed with glucose and the daily dosage added to the grain fed. It appeared to be quite palatable in this form. Hard pellets of diethylstilbestrol dipropionate weighing 100 mg. were implanted subcutaneously behind the shoulder through an incision in the skin.[1]

The anterior pituitary extract used was an initial extract prepared by A. J. Bergman of this laboratory. It contained 4000 I.U. of lactogen per gram, 3000 chick units of thyrotropin, 1000 chick units of gonadotropin, 250 guinea pig units of blood-sugar-raising principle and 100 chick units of adrenotropin as assayed by Mr. Bergman.

The goats in this experiment were on dry feed at all times consisting of alfalfa hay and a mixed grain ration. They were milked twice daily.

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[1] The diethylstilbestrol powder was contributed by Merck and Co. and the pellets by the Blue Line Chemical Co., St. Louis.

COMPARISON OF DIFFERENT METHODS OF ADMINISTRATION

We have already reported that subcutaneous injection of diethylstilbestrol was effective in initiating and maintaining lactation in goats (29, 30). This method of administration has perhaps less practical application than others such as percutaneous application to the udder, implantation of pellets or especially oral treatment We therefore wished to compare the effectiveness of these different methods of administration in goats.

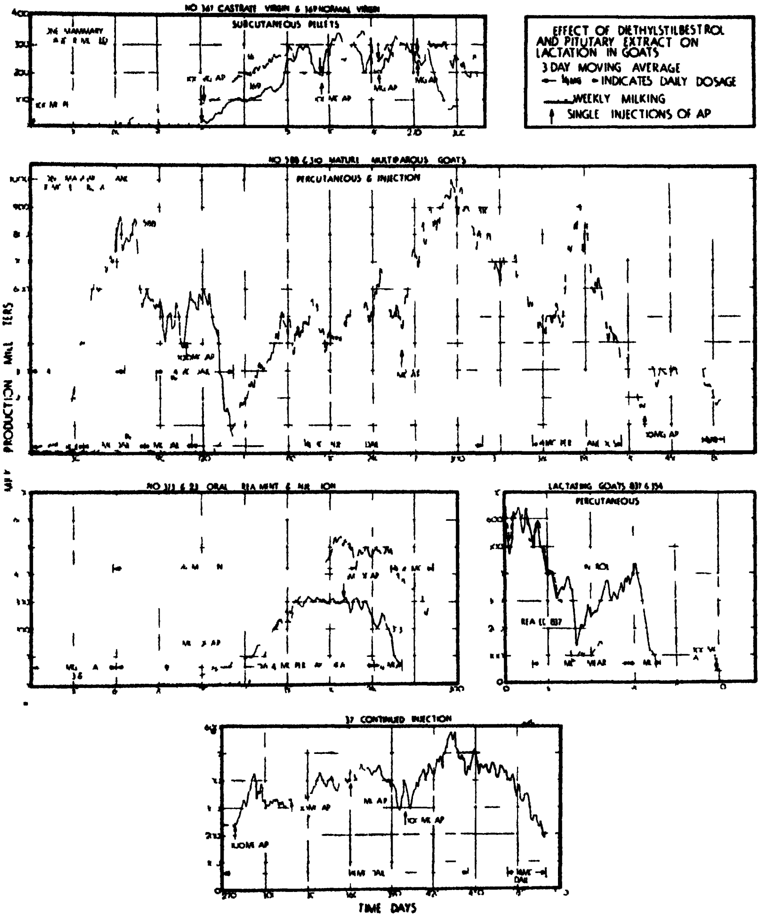

Fig. 1. Effect of diethylstilbestrol and pituitary extract on lactation in goats.

Administration of hormones by *subcutaneous injection* is probably the most common means used for laboratory animals and the method first used by us in giving diethylstilbestrol to goats. Besides the four goat lactations previously reported, two additional lactations have been secured from goats by injection of diethylstilbestrol. Goat #310, which had failed to respond to percutaneous application, readily came into production on ¼ mg. per day by injection and reached a production of a liter a day after a single dose of anterior pituitary extract (fig. 1). No. 231, a virgin yearling goat, produced over 500 ml. of milk a day on a similar injection dosage after failing to respond to oral treatment. Also we previously reported eight months of lactation from #371 given the same dosage (29, 30). She continued to lactate for seven more months and with periodic injections of anterior pituitary extract she recovered to her previous peak of production, *i.e.*, 600 ml. (fig. 1).

Administration of diethylstilbestrol by subcutaneous injection appears to be an efficient method of initiating lactation in goats. A very low dosage was required. The labor of treatment could be reduced by giving two or three injections a week instead of daily.

Percutaneous treatment of laboratory animals with estrogens has been shown to be efficient in causing genital changes (53, 10, 42) and mammary gland growth (47, 33, 24). We found that 0.01 mg. of diethylstilbestrol applied daily to the shaved skin would cause extensive mammary gland growth in male rabbits in 30 days (32).

Percutaneous inunction of the udder is a convenient method of administration of diethylstilbestrol. This is the method used by Folley *et al.* (17, 21) who treated the udders of goats three times a week giving a total of 30 mg. We found that 7 and 14 mg. per week were ineffective in a mature dry goat (#310) but 28 mg. promptly brought a second goat (#588) into lactation. Four milligrams daily applied to the udder favorably affected the established lactation of #310 but 10 mg. had little effect on the lactation of a cow (28). One-quarter milligram daily by injection caused copious lactation in the first goat and in several others. According to these results on a dosage basis injection of diethylstilbestrol is much more efficient than percutaneous administration in causing lactation in goats. Eight times our usual injection dosage did not cause lactation while 16 times the subcutaneous dosage was effective.

Oral administration of a drug has considerable advantage over other methods for clinical or practical use in farm animals. In comparison to the subcutaneous dosage diethylstilbestrol has been shown to be comparatively much more active orally on the genital organs and mammary glands than are the natural estrogens in laboratory animals (30, 27, 41) and in primates (35, 23, 7).

Oral administration for other purposes than milk secretion has been shown to require about five times the dosage of diethylstilbestrol as by injection. When we gave this amount (1.25 mg. daily) to three goats (#373, #355, #231) no lactation response was secured for 50 to 123 days. No. 355 gradually began to lactate when raised to 2.5 mg. daily and responded to anterior pituitary extract. However, #375 responded to an anterior pituitary extract injection with lactation when being given only 0.625 mg. per day (fig. 1). Ten times the injection dosage to a fourth goat (#360) had no effect, but she began to lactate when 5 mg. were given daily. These three goats which finally lactated on oral treatment produced a maximum of about 300 ml. of milk daily. From our previous results we had expected them to exceed 500 ml. Indeed one of these goats (#373) had lactated for six months with a maximum of 550 ml. under diethylstilbestrol injections the previous year. One of the three goats (#231) which did not respond to 5 times the injection dosage reached 575 ml. of milk per day when given ¼ mg. daily injections.

Why these goats did not respond better to oral dosage is uncertain. It may be that diethylstilbestrol is destroyed in the rumen and if placed directly in the reticulum the treatment might have been more effective. These goats may have been more recalcitrant to diethylstilbestrol treatment than usual and would have responded to anterior pituitary extract injection sooner. Folley and Young reported such a case (21). In a trial of the effect of diethylstilbestrol on the chemical enrichment of milk produced by dairy cows Folley *et al.* (19) also found that oral administration did not have the expected effect.

Subcutaneous implantation of pellets is the mode of administration of diethylstilbestrol involving the least labor. The continuous absorption of the hormone makes for more efficient use, and it has been shown to be quite effective (12, 23). MacBryde *et al.* (34) reported that on a dosage basis pellets were 5 to 10 times as effective as injections in castrate women. The effect of 100 mg. pellets lasted for 400 to 800 days. From 0.127 to 0.25 mg. was absorbed daily. We used the dipropionate form in this case which is somewhat more slowly dissolved.

Two virgin yearling goats (#369, #367) each of which had a 100 mg. subcutaneous pellet of diethylstilbestrol dipropionate came into milk production after a long latent period. With the addition of anterior pituitary extract at 25–35-day intervals production rose to 350 ml. (fig. 1). This greatly exceeded the production secured the previous year from these goats when treated by injection. This was in spite of the removal of half of the udder after the previous lactations. It seems probable that the long latent period could have been shortened by the earlier administration of anterior pituitary extract. As will be shown in another report, considerable growth of the mammary glands occurred. It appears probable that implantation of pellets or crystals of diethylstilbestrol is a very efficient method of securing milk production from virgin or dry dairy animals especially in combination with anterior pituitary extract.

EFFECTS OF ANTERIOR PITUITARY EXTRACT INJECTION

Anterior pituitary extracts have been shown to be effective in bringing into lactation laboratory animals with prepared mammary glands (51).

Folley (21) reported a case of a goat which did not come into milk production on diethylstilbestrol administration until anterior pituitary extract had also been given. In addition there have been several reports that anterior pituitary extract injections will increase milk production in lactating cows (4, 20), and women with deficient lactation (26, 45, 25). Sykes *et al.* (49) obtained good results in cows with a fat metabolism extract of anterior pituitary but poor results with a lactogenic extract.

Single injections of anterior pituitary extract seemed to have an activating effect on lactation in diethylstilbestrol-treated goats. Instead of there being a brief rise in production, production tended to improve for some days after the injection. The time from injection to the subsequent decline in production was 62, 43, and 47 days with successive injections in #367. In the case of #369, peaks occurred 67, 18, 10, 7 days after injection. With #310 production did not decline for 37 and 38 days after single injections of anterior pituitary extract. In the case of #371, production continued to rise for 13, 25, 20 and 36 days after anterior pituitary extract injections.

The amount of increase in production from anterior pituitary extract injections was gratifying in most cases. The best percentage increases in the case of goats already producing fairly well were 100 per cent (#367); 110 per cent (#369); 90 per cent (#371) and 150 per cent (#310). Other responses were less. No. 369 responded better to 100 mg. injections than to 77 mg.; 1 mg. per pound of body weight. No. 373 did not respond at all to such an injection.

An initial extract of anterior pituitary proved to be a valuable addition to diethylstilbestrol therapy of goats. We previously reported the use of a more purified lactogenic extract of anterior pituitary under similar conditions (29, 30). Lactogenic extract did not prove to be as effective as an extract with 95 per cent of the inactive protein removed but containing large amounts of other anterior pituitary hormones such as thyrotropin, adrenotropin, blood-sugar-raising principle, gonadotropin and possibly other unassayed factors. This is in agreement with the results of other investigations on the effects of anterior pituitary extracts on milk production (4, 20). Although lactogen is the principal hormone controlling initiation and maintenance of lactation other anterior pituitary factors act synergistically with lactogen in the galactogogic effect (5). Thyrotropin especially, acting through the thyroid gland, causes an increase in metabolism of cells and in quantity of milk production. Since administration of estrogens causes a great increase in lactogen output of the pituitary; lactogen should not have been a limiting factor in these lactations.

Further evidence that the response to anterior pituitary extract observed in this study was not a response primarily to lactogen is provided by a study of the dosages administered. To bring a pseudo-pregnant rabbit into good lactation requires 260 McShan-Turner units of lactogen or 33 McShan-

Turner units per pound body weight given in six daily injections (6). At this rate a 75-pound goat should require 2475 McShan-Turner units in proportion to body weight. This amount of lactogen was contained in 495 mg. of the lactogenic extract used previously or in 614 mg. of initial anterior pituitary extract used in this study. The lactogenic extract was given at the rate of one milligram per pound of body weight per day for 6 to 12 days. The response was almost immediate in three cases and lasted little beyond the period of injections. Averaging the milk production for 10 days before and after these injections the increase in production amounted to 6 and 14 per cent (#373), 19 and 1 per cent (#371). However, when a single 100 mg. injection of initial anterior pituitary extract was given the increase in production was as high as 150 per cent and continued for as long as 30 to 40 days. Thyrotropin has a prolonged effect on metabolism in some cases, but it also affects the heart rate (43). The pulse rate of five of these goats was recorded daily for 48 days after a single injection of 100 mg. of anterior pituitary extract. There was no apparent effect in four cases in which good lactation response was secured. The pulse rate of the fifth goat increased from 93 to 136 beats per minute and remained above 100 for 41 days. This goat did not increase in milk production in response to the anterior pituitary extract injection. Probably the response to initial anterior pituitary extract was due to a combination of the various pituitary factors contained therein.

Administration of estrogen may not increase the production of other pituitary hormones as it does that of lactogen (46, 39). By supplying a whole extract of anterior pituitary, we were increasing the supply of other pituitary hormones needed for increased milk production.

THE EFFECT OF NON-CONTINUOUS ADMINISTRATION OF DIETHYLSTILBESTROL

Walker and Stanley (52) reported that intermittent treatment with diethylstilbestrol dipropionate could be used to cause further increases in milk production in a virgin cow which had been brought into production with injections of the hormone.

We have tried the effect of stopping treatment after a peak of production had been reached and a decline had set in in 9 cases. Stopping treatment generally seemed to have the effect of terminating the decline in production in most cases within one to seven days (#360, #355, #231, #427,[1,2] #371, #373). Production either returned to normal (#360, #355, #231) or increased somewhat for 2 to 10 days (#588, #427,[1,2] #371, #373). A decline again set in after 10 to 25 days (#588, #427,[1,2] #371, #373). No effect of stopping treatment was observed in one case (#310[1]). Production continued to decline for 24 days in another (#310[2]).

When treatment was resumed after 22 to 72 days the results were variable. Production tended to level off after a latent period of 1 to 12 days

(#231, #427, #310^{1,2}) and then dropped in three cases (#231, #310^{2}, #427), increased for 2 days then slowly declined (#355), dropped and then recovered (#360, #588) or had no effect (#371).

A decline in production from diethylstilbestrol treatment may occur after a time due perhaps to the accumulation of excess hormone or to loss of its effectiveness. If treatment is then terminated production may plateau or even recover somewhat and then again begin to decline. Resumption of daily treatment at this time was not usually successful in holding up production for long. Injection of anterior pituitary extract was much more efficacious.

EFFECT OF DIETHYLSTILBESTROL ON PARTURIENT GOATS

It was considered that the effect of diethylstilbestrol on normal parturient goats already in milk should be investigated. If this chemical were capable of increasing the milk production of such dairy animals, it would be of considerable practical advantage. There are many reports, however, that estrogens will inhibit lactation as well as reports to the contrary (see 36 for review). Folley (14) reported that estradiol benzoate and estrone caused a reduction of 20 per cent in milk production of cows but the milk was richer in fat and solids not fat. Diethylstilbestrol implanted subcutaneously or injected into lactating cows did not cause any reduction in milk volume but increased the proportion of solids in other cases (15, 48).

Whether or not estrogens cause a reduction of milk production in lactating animals is perhaps a question of dosage. High dosage will certainly inhibit lactation. We were interested in learning what dosages of diethylstilbestrol similar to those which caused lactation in dry goats would do to milk production in normal parturient goats. We have already reported the inconclusive results secured in two such cases (29, 30).

Two additional parous goats which were already in milk were treated by percutaneous application of diethylstilbestrol. No. 351 served as the control at first and #837 was given 1 mg. per day. Both goats declined rapidly in production due probably to a spell of cold weather (November). The control goat then recovered to 440 ml. while the production of the treated goat only rose to 180 ml. during the next month. The control goat (#351) was then given twice the dosage of diethylstilbestrol. Production promptly plunged from 430 ml. to 40 ml. in 15 days where it was maintained during the next 50 days in spite of termination of treatment. An injection of 100 mg. of anterior pituitary extract had no effect.

The administration of diethylstilbestrol to parturient goats was at least of no obvious advantage on volume of milk production from mammary glands already in secretion. The early decline in production of #837 when diethylstilbestrol was applied to the udder would have looked like a clear case of suppression of lactation if the control goat had not declined similarly.

The poor recovery of #837 may or may not have been due to the treatment. The response of #351 might be considered a clear-cut case of suppression of lactation except that the dosage used (2 mg.) had proven inadequate for the initiation of lactation in a dry goat (#310). Twice that dosage readily brought a second goat (#588) into heavy lactation and when used later on #310 in the declining phase of her lactation it caused a precipitous, though temporary, rise. That a certain dosage of diethylstilbestrol would bring dry goats into long sustained lactation and yet would cause normal parturient goats to dry up does not appear rational in the present state of our knowledge.

EFFECT OF DIETHYLSTILBESTROL TREATMENT ON THE FUTURE BREEDING HISTORY

The effect of diethylstilbestrol on the subsequent reproductive ability of treated animals is of considerable practical interest. If no permanent deleterious influence is to be expected, diethylstilbestrol might be useful in such cases as when goats, which have a seasonal breeding period, have failed to conceive. Thus, instead of losing a year's production, these open goats could be made to pay for their keep. There would also be less prejudice against diethylstilbestrol treatment of cows which have failed to breed but which might eventually recover. According to Allen *et al.* (1), many descriptions of ovaries following estrogen treatment have reported inhibiting or depressing effects on follicular development. This probably occurs through a depressing effect on the follicle-stimulating hormone of the anterior pituitary. If the hormone treatment is not too severe, the depression may be temporary and the ovaries may recover fully soon after treatment is stopped.

Most of the diethylstilbestrol treated goats in this experiment were under continuous treatment for long periods of time. On the other hand, the amount of chemical administered was kept as low as was practical. Under these conditions most of the goats showed cyclic estrus; that is, there were periods during which the characteristic signs of estrus were especially prominent and they would accept the buck more readily. At these times milk production tended to decline temporarily. Similar cyclic response to continuous estrogenic treatment has been reported in laboratory animals (8).

The ovaries of these diethylstilbestrol treated goats were examined at autopsy after termination of the treatments. The ovaries from #371, which was under almost continuous diethylstilbestrol treatment for 428 days, were definitely abnormal. They appeared to have degenerated until normal ovarian tissue was absent. On the other hand, the ovaries from #310 were normal in appearance at autopsy weighing 5.46 gm. and containing several large follicles. No. 310 had had a rest period of 72 days without treatment shortly before autopsy which might have allowed some recovery to occur. No. 371 also had a 29-day rest period. The ovaries of #231, #373 and

#355 were in an essentially normal although quiescent condition while one ovary of #360 had a corpus luteum. No. 369, which had had a series of 96 daily injections and then a subcutaneous pellet for 331 days, a total of 427 days, had ovaries which weighed 12.7 g. and contained several 2 cm. follicles. These ovaries would undoubtedly have recovered promptly on cessation of treatment.

Two normal parturient goats (#370, #443) had been given daily injections of diethylstilbestrol for 20 and 107 days respectively. They were rested 49 and 157 days and then were given 5 to 6 ml. injections of pregnant-mares-serum extract (Gonadin[2]) at 16-day intervals. No. 370 bred after each of three treatments but apparently did not conceive. No. 443 conceived after the second treatment (April) and bore a rather small but otherwise normal kid.

Thus in only one case after 15 months treatment with diethylstilbestrol was an abnormal condition of the ovaries found. The ovaries of three goats were quiescent with very small follicles. One goat had large follicles; a second had normal sized follicles; a third had a corpus luteum indicating that ovulation must have occurred. That such ovaries were capable of recovering their normal function is shown by the response of two goats to gonadin treatment.

THE EFFECT OF DIETHYLSTILBESTROL IN PREGNANT GOATS

There have been many reports of the termination of pregnancy by administration of estrogens (1). This is a dosage problem for small dosages may not affect pregnancy. The greater the interval between fertilization and initial treatment the larger the amount of estrogen tolerated. Abortion has been reported in sheep within 1 to 8 days of the injection of 20,000 international benzoate units (3). Folley and Scott-Watson (16) reported abortion in two of four cows in late pregnancy after 13 inunctions of the udder with 2.9 gms. of diethylstilbestrol dipropionate in 31 days.

Our results with two goats do not substantiate this. The udder of goat #443 was smeared with 4 mg. per day of diethylstilbestrol from the 63rd to the 136th day of pregnancy and during the last 6 days. A normal kid was produced on the 149th day of pregnancy. A second goat (#360) was fed 1.25 mg. a day of diethylstilbestrol for the last 73 days of pregnancy and kidded normally.

The dosage of diethylstilbestrol administered by Folley and Scott-Watson was 1.5 times that which we gave goat #443 on a body weight basis (assuming 1000-lb. cows). Possibly if the dosage to these goats had been several times greater, abortion would have resulted. However, dosages high enough to initiate milk secretion in dry goats did not cause abortion.

[2] Contributed by the Cutter Laboratories.

THE EFFECT OF DIETHYLSTILBESTROL ON THE BODY WEIGHT OF GOATS

An adverse effect of diethylstilbestrol on body weight of laboratory animals has been reported (40). Six to 10 mg. subcutaneous pellets caused a daily loss in body weight of 0.3 to 3.6 gm. in male and female rats (22). As low a dosage as 2 to 3 mg. a day caused a slight but definite inhibition of the growth of male rats (41).

The body weights were available for nine of these goats taken at the beginning of long periods of treatment with diethylstilbestrol and again at termination (table 1). The periods of treatment were from 9.5 to 17 months. Seven young female goats and two males gained from 20 to 43 pounds during the treatment and were of essentially normal size for their age. The goats were in good health during the entire time. They appeared thrifty and ate well. One mature goat (#310) was thin for some time during her treatment and would not consume sufficient grain to gain weight. That this was due to the diethylstilbestrol treatment is doubtful. The two males listed had 200 and 277 mg. pellets of diethylstilbestrol dipropionate implanted subcutaneously.

TABLE 1

Gain in body weight of goats during continuous diethylstilbestrol treatment

Number	Date	Body weight	Termination		Length of treatment	Gain in body weight
			Date	Body weight		
		lbs.		*lbs.*	*mos.*	*lbs.*
231	11/40	48	9/41	72	10.7	24
360	3/41	62	10/41	82	6.7	20
355	12/40	38	10/41	64	9.5	26
373	4/40	48	10/41	71	15.3	23
371	7/40	75	9/41	98	15.0	23
367	7/40	47	10/41	90	14.3	43
369	7/40	42	9/41	78	13.3	36
43 ♂	11/40	32	9/41	66	11.0	34
833 ♂ Castrate	4/40	86	9/41	113	17.0	25

SUMMARY

Administration of diethylstilbestrol by subcutaneous injection, orally, by inunction or diethylstilbestrol dipropionate by implantation of pellets caused copious and prolonged lactation in virgin and dry goats. This is significant because of the ability of estrogens to increase the anterior pituitary lactogenic output. One-quarter milligram per day was sufficient by injection. Oral administration appeared to be least effective up to 5 mg. per day or 20 times the subcutaneous dose. Inunction of the udder required 4 mg. daily or 16 times the subcutaneous dose. Anterior pituitary extract was effective in augmenting the production caused by diethylstil-

bestrol by supplementing the increased lactogen output with other anterior pituitary factors or in initiating lactation in diethylstilbestrol-treated goats. Stopping daily treatment was sometimes effective in improving production for a time but restarting treatment had little effect. Hormone treatment of normal parturient goats was not beneficial on volume of production. Treatment during the last half of pregnancy had no deleterious effect in two cases. Ovaries were essentially normal after long periods of treatment with one exception. Two goats were bred out of season after termination of diethylstilbestrol injections. One conceived and bore a kid. No adverse effect on growth or health of the goats from diethylstilbestrol treatment was observed.

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THE FAT METABOLISM OF THE MAMMARY GLAND OF THE NORMAL COW AND OF THE COW IN KETOSIS

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Evidence was presented in previous publications (31, 33) that sufficient blood fat is taken up by the active mammary gland of the cow to account for most of the milk fat. More recent publications (24, 28) from this laboratory demonstrated for the first time the utilization of relatively large quantities of the intermediate product of fat metabolism, β-hydroxybutyric acid, by the mammary gland of the cow. In ketosis it was observed that this utilization was increased by over 100 per cent and would account for the total oxygen consumption of the gland if oxidized for energy purposes. The acetoacetic acid and acetone fraction was not used in measurable quantities by the gland of either the normal cow or the cow with ketosis. As it is generally assumed that most of the tissues of the body utilize both β-hydroxybutyric acid and acetoacetic acid for energy purposes it was considered possible that β-hydroxybutyric acid was used in direct synthesis, and the suggestion was made that some of the short chain fatty acids peculiar to milk fat were derived from this source (27).

In this paper data are presented on the effect of ketosis and glucose therapy in ketosis upon the character of the fatty acids synthesized by the mammary gland.

Data are also presented on the source of energy for the mammary gland and on the cause of the low respiratory quotient during fasting. The volume of blood per unit of volume of milk was calculated by a method less open to criticism than methods used previously.

METHODS

The following methods of analysis were used: blood glucose, the Somogyi (23) modification of the Shaffer-Hartmann method; blood lactic acid, the method of Miller and Muntz (19), as modified by Koenemann (16) and Barker and Summerson (2); blood acetone bodies, the method of Barnes and Wick (3); blood calcium, the Clark-Collip modification (5) of the Kramer-Tisdall method; blood acid-soluble phosphorus, the method of Fiske

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and Subbarow (7); blood amino acids, the Folin method (8); blood fat, a modification (25) of the Allen (1) method; blood oxygen and carbon dioxide, the method of Van Slyke and Neill (37); hemoglobin, Evelyn and Malloy (6); milk calcium and phosphorus, Morris, Nelson, and Palmer (20); lactose, the method of Bierman and Doan (4). Milk fat was determined by the Babcock method. The various milk fat constants were determined by the methods approved by the Association of Official Agricultural Chemists. The iodine value was determined by the Hanus method. Arterial and mammary venous blood samples were taken as previously described (26, 31).

EXPERIMENTAL

An examination was made of the effect of severe clinical ketosis, chronic ketosis, and the administration of large quantities of glucose in ketosis upon the type of fatty acids in the milk fat as measured by the various fat constants.

Table 1 contains a summary of the results. In severe clinical ketosis characterized by ketonemia, hypoglycemia and varying degrees of inanition the Reichert-Meissl, Polenske, and saponification values of the milk fat were considerably below normal and the iodine values were elevated markedly. In less severe ketosis and chronic ketosis characterized by ketonemia and hypoglycemia but differing from the above in that the food intake was normal, the short chain fatty acids were present in more nearly normal quantities and the degree of unsaturation was much less.

Two cows, Pa–1 and N–34, were treated for severe ketosis by pumping 6 lbs. of glucose into the rumen. Blood and milk samples were obtained prior to and for the first few days following treatment. The administration of glucose produced a marked increase in blood glucose and lactic acid for the following 2 days and a decrease in blood ketone bodies. Within 36 to 48 hours the short chain fatty acids of the milk fat increased significantly as shown by the saponification values of the milk fat of animal Pa–1 and the Reichert-Meissl and saponification values of the milk fat of animal N–34. The appetite of N–34 improved immediately and the increase in the short chain fatty acids could have been due in part to the increased intake of food substances other than glucose. The increase in the short chain fatty acids of the milk fat of animal Pa–1, however, appeared to be due directly to the glucose therapy as the animal was almost completely off feed for the first 2 days following treatment.

It was expected that the degree of unsaturation of the fatty acids of the milk fat would decrease with the increase in the percentage of the short chain fatty acids following glucose therapy. Instead there was an increase in the iodine numbers of the milk fat of both animals.

While the Reichert-Meissl values increased shortly after glucose administration in animal N–34, the Polenske value was not materially affected.

TABLE 1

The effect of ketosis and glucose therapy in ketosis upon the character of the fatty acids of milk fat

Cow	Date and time	Blood substances			Milk fat constants				Remarks
		Acetone bodies (as acetone)	Glucose	Lactic acid	Reichert-Meissl value	Polenske value	Saponification value	Iodine value	
		mg.%	*mg.%*	*mg.%*					
P–1	2– 3–41	53.7	20.9	5.0	22.3	1.5	213.5	46.8	Eating small quantity of hay only
	2– 7–41	15.6	36.0	3.6	25.6	1.7	216.4	46.7	Eating hay and some grain
	2–19–41	3.0	42.0	7.4	27.2	1.4	223.4	40.4	Normal appetite
	3–11–41	5.1	45.5	8.3	27.4	2.9	227.2	38.5	Normal appetite
W–1	3–15–41	45.1	15.5	10.5	18.0	0.9	212.4	46.4	Very poor appetite
	4–19–41	1.5	33.0	5.8	31.6	2.9	229.3	34.8	Normal appetite
Pa–1	5–13–41 A.M.	18.5	18.2	3.0			219.4	40.6	Very poor appetite. Pumped 6 lbs. glucose into rumen
	P.M.						219.4	41.0	
	5–14–41 P.M.	4.0	41.5	30.0			223.2	41.5	Very poor appetite
	5–15–41 A.M.	17.4	59.0	30.0			223.4	42.3	Very poor appetite
	5–16–41 A.M.						223.0	42.5	Appetite improved
N–34	2–18–41 A.M.	67.3	15.5	5.0	25.4	2.1	215.6	48.2	Very poor appetite. Pumped 6 lbs. glucose into rumen
	2–19–41 A.M.	18.5	46.3	26.0	25.0	2.5	214.8	47.9	Appetite improved
	2–20–41 A.M.	13.7	42.7	10.2	28.9	2.3	218.8	51.4	Appetite improved
	3–11–41	33.5	25.0	6.4	33.4	2.8	228.0	41.1	Normal appetite, chronic ketosis
	4–19–41	13.2	33.5	6.7	35.2	3.8	230.9	35.9	Normal appetite chronic ketosis
	5– 9–41	46.1	29.2	7.2	28.5		226.3	42.7	Normal appetite chronic ketosis

Likewise, the Polenske value did not increase in the case of animal P–1 until several weeks after the Reichert-Meissl value returned to normal.

DISCUSSION

Previous work from this laboratory (26, 30) indicated that not only β-hydroxybutyric acid but also other fat may be oxidized by the mammary gland. In ketosis it was found that the gland used much more β-hydroxybutyric acid than the gland of the normal cow. As the short chain fatty acids of milk fat are lowered markedly in ketosis it is apparent that these fatty acids are not derived from β-hydroxybutyric acid. It is probable, therefore, that this substance is oxidized by the gland for energy purposes.

It is significant that the amount of β-hydroxybutyric acid utilized by the gland in ketosis would require practically all of the oxygen taken up by the gland for complete combustion, which indicates that all of the energy for the gland of the cow with severe ketosis may be derived from the oxidation of β-hydroxybutyric acid. If this hypothesis is correct it would be expected that there would be a rather constant maximum utilization in ketonemia in which the quantity of β-hydroxybutyric acid taken up by the gland would correspond to the energy requirements of the gland. In severe ketonemia where a large excess of β-hydroxybutyric acid is available, we have found that the gland uses approximately twice as much of this substance as the gland of the normal cow and that this utilization remains approximately equal to but not significantly in excess of the amount of oxygen available for its complete combustion. If this is confirmed by work now under way, it may be possible to make direct calculations of the total energy requirements of the gland.

Since the gland in ketosis was found to utilize approximately the same quantity of glucose as that of the normal cow (34), it was suggested that the increased utilization of β-hydroxybutyric acid was due to a shift from the oxidation of β-hydroxybutyrate and other fat of the gland to the sole oxidation of the former substance by the gland in ketosis. Assuming that this suggestion is valid, it should be pointed out that if the short chain fatty acids of milk fat are derived from the oxidation and subsequent reduction of the longer chain fatty acids, the former would decrease in ketosis. In fact, if the hypothesis suggesting a shift from the oxidation of other fat to the sole oxidation of β-hydroxybutyrate is correct, the failure of the short chain fatty acids to decrease in ketosis would represent a serious objection to the theory (13, 14) that these fatty acids are derived from the longer chain fatty acids.

The data in table 1 shed considerable light upon this problem. It will be observed that the short chain fatty acids are lowered considerably in severe clinical ketosis. However, these animals were consuming feed at sub-maintenance levels and the effect may have been due to fasting which is known to produce a decrease in the short chain fatty acids. Observations were made on animal W–1 on 4–19–41, and on animal N–34 (a case of chronic ketosis) on 3–11–41, 4–19–41, and 5–9–41. It will be noted that the fatty acids of the milk fat approached normal at the time of these observations. An increased utilization of β-hydroxybutyrate by the mammary glands on these dates would be expected on the basis of previous work (31), as the blood ketone bodies were quite high. That such was the case was borne out by an arteriovenous experiment on animal N–34 on 5–9–41 showing a utilization of 5.0 mg. % of β-hydroxybutyric acid, which is a little more than double the average normal utilization (28). If there is a decrease in the oxidation of fatty acids other than β-hydroxybutyric acid by the mammary gland of the cow with ketosis, as suggested, the above findings represent a serious objection to the view that the short chain fatty acids are derived from the oxidation and subsequent reduction of the longer chain fatty acids.

It is possible, of course, that the increased utilization of β-hydroxybutyric acid by the gland in ketosis may be associated with a decreased oxidation of carbohydrate or protein. As some deamination appears to take place in the gland (11, 29) the latter possibility must be entertained. Due to the limited amount of carbohydrate which has been shown to be available to the gland for other than lactose synthesis the suggestion that any such quantity of glucose undergoes oxidation in the gland appears unwarranted. The possibility of use by the mammary gland of carbohydrate or protein for energy purposes will be examined more fully in a later communication.

The data in table 1 also have some bearing upon the question of the possible synthesis of fat from carbohydrate in the mammary gland. It will be noted that the blood sugar values of the four cows were extremely low when the initial samples were taken, ranging from 15.5 to 20.9 mg. per cent. The short chain fatty acids of the milk fat were also lowest at this time. The gradual increase in the blood sugar in animal P–1 from 20.9 to 45.5 mg. per cent was accompanied by a gradual increase in the percentage of short chain fatty acids. Even more striking was the sudden increase in the short chain fatty acids following the administration of large quantities of glucose to animals Pa–1 and N–34. The Reichert-Meissl, Polenske, and saponification values of the milk fat of W–1 on 3–15–41 were the lowest of all the four animals. The blood glucose of this animal had been below 20.0 mg. per cent for several days prior to the taking of the samples. The appetite of this animal was also extremely poor during this period. The results strongly favor the possibility of the synthesis of some of the short chain fatty acids of milk fat from carbohydrate.

Previously it had been reported by Graham *et al.* (10) that the respiratory quotient of the active mammary gland of the goat exceeded unity. This has been confirmed by Shaw and Petersen (32) on cows and Reinecke *et al.* (21) on goats. The latter workers reported that the respiratory quotient of the mammary gland of the fasted goat was less than unity. This is confirmed on the cow by the data in table 2. The animal was fasted for a period of 54 hours. Arteriovenous samples were drawn at the end of 48, 53, and 54 hours. The milk production of the animal was eight pounds per day at the beginning of the experiment, and declined to approximately one pound at the time the blood samples were taken. It will be observed that the utilization of amino acids, glucose, and fat was extremely small. The failure of the gland of the fasted animal to utilize amino acids has been reported previously by Reinecke *et al.* (22). It is possible that this cessation of the utilization of amino acids may be associated with the decline in the lower acids of milk fat during fasting. Since the R. Q. of the gland of the fasted cow was less than unity it was considered possible that there would be an increased oxidation of fat other than β-hydroxybutyric acid for energy purposes, assuming that the normal gland oxidizes other fat. The formation of acetoacetic acid would constitute proof of such oxidation. However, there was no significant production of this substance. The failure to observe the production of acetoacetic acid does not, however, preclude the possibility of the oxidation of fat other than β-hydroxybutyric acid by the gland. However, β-hydroxybutyric acid continued to be used at approximately half the normal rate. If the latter substance is oxidized for energy purposes, it may account, to a large extent, for the low respiratory quotient of the mammary gland of the fasted cow, as the complete oxidation of β-hydroxybutyric acid would result in an R. Q. of 0.89. The oxidation of this substance would also tend to depress the respiratory quotient of the gland of the normal cow. As the R. Q. exceeds unity in the latter case, considerable synthesis of oxygen-poor from oxygen-rich substances may occur.

TABLE 2

The effect of fasting upon the utilization of various blood substances by the mammary gland of the cow

Period of fasting	Blood conc. change	Glucose	Fat	Amino acids	Acetoacetic acid and acetone	β-hydroxy-butyric acid	R. Q.
hrs.	*%*	*mg.%*	*mg.%*	*mg.%*	*mg.%*	*mg.%*	
48	0.00	−4.8	−1.2	0.00	+0.17	−1.22	0.76
53	0.33	−1.5	−0.10	0.04	+0.06	−1.07	0.88
54	0.68	−0.8	−0.02		+0.11	−0.79	0.87

We have observed that the feeding of cod liver oil, which produces a marked decrease in the short chain fatty acids, also results in a low respiratory quotient (27). The decline in the short chain fatty acids which we observed following glucose administration (15, 27) appears to have been due to the animals going off feed, as our more recent work (15) has shown that the feeding of large quantities of glucose in addition to the usual ration does not materially affect the various fat constants.

There are a number of serious objections to the hypothesis that milk fat is synthesized from carbohydrate and definite conclusions will have to await the results of further investigation. The fact that the gland uses sufficient blood fat to account for practically all of the milk fat (31) cannot be ignored. Further work bearing upon this aspect of the problem is summarized in table 3. In these experiments the volume of blood per unit of volume of milk was calculated on the basis of the arteriovenous differences of calcium and phosphorus, and the total amounts secreted in the milk in

TABLE 3

Mammary gland balance

Blood precursor	Gland utilization per liter of blood	Suggested end product		Liters of blood required	Liters of milk produced	Ratio of blood volume to milk volume	Per cent of milk substance accounted for
		Milk substance	24-hr. production				
	gm.		*gm.*				
Expt. 1							
Calcium	0.0023	Calcium	28.50	12404.5	25.30	493–1	...
Phos.	0.0024	Phos.	20.09	8370.8		331–1	
Ca & P	0.0047	Ca & P	48.59	10338.5	...	408–1	...
Fat	0.0646	Fat	787.0	12182.7	...	482–1	84.6
Glucose	0.1000	Lactose	1236.0	12364.6	...	489–1	81.4
β-OHBut.	0.0089	...	...	...	...		...
Arteriovenous blood concentration change: 0.69 per cent.							
Expt. 2							
Calcium	0.0016	Calcium	17.69	11056.3	17.58	629–1	
Phos.	0.0022	Phos.	15.88	7218.2		411–1	...
Ca & P	0.0038	Ca & P	33.57	8834.2		502–1	...
Fat	0.0618	Fat	507.1	8205.8		467–1	107.9
Glucose	0.1010	Lactose	880.4	8762.0	...	496–1	101.2
β-OHBut.	0.0167	...	...	...	...	...	...
Arteriovenous blood concentration change: 0.00 per cent.							
Expt. 3							
Calcium	0.0020	Calcium	21.62	10810.0	17.71	610–1	...
Phos.	0.0020	Phos.	18.10	9050.0		511–1	
Ca & P	0.0040	Ca & P	39.72	9930.0		561–1	...
Fat	0.0674	Fat	720.3	10687.2	...	603–1	93.3
Glucose	0.1180	Lactose	915.8	8608.6	...	486–1	115.4
β-OHBut.	0.0147		...	...	...		...
Arteriovenous blood concentration change: 0.00 per cent.							
Expt. 4							
Calcium	0.0025	Calcium	21.62	8648.0	17.71	488–1	...
Phos.	0.0021 –	Phos.	18.10	8657.1		489–1	...
Ca & P	0.0046	Ca & P	39.72	8652.0	...	488–1	...
Fat	0.0724	Fat	720.3	9949.2	...	562–1	86.8
Glucose	0.1220	Lactose	915.8	7506.7		424–1	115.3
β-OHBut.		...	...	...		...	...
Arteriovenous blood concentration change: 0.47 per cent.							
Expt. 5							
Calcium	0.0019	Calcium	23.13	12173.7	18.72	650–1	...
Phos.	0.0020	Phos.	15.87	7935.0		424–1	...
Ca & P	0.0039	Ca & P	39.00	10000.0		535–1	...
Fat	0.0760	Fat	802.9	10560.8		564–1	94.8
Glucose	0.1200	Lactose	978.9	8157.3		546–1	97.9
β-OHBut.	0.0178	...	...	...	...	...	...
Arteriovenous blood concentration change: 0.00 per cent.							
Summary							
Calcium	0.0021	Calcium	22.52	10931.1	19.40	563–1	...
Phos.	0.0021	Phos.	17.61	8246.2	...	425–1	...
Ca & P	0.0042	Ca & P	40.13	9588.6	...	494–1	...
Fat	0.0684	Fat	707.5	10317.1	...	532–1	92.8
Glucose	0.1122	Lactose	985.5	9070.7	...	468–1	105.5
β-OHBut.	0.0145	...	...	...	...	...	...
Arteriovenous blood concentration change: 0.23 per cent.							

a period of 24 hours. Determinations were also made of the utilizations of glucose, fat, and β-hydroxybutyric acid. The calcium, phosphorus, and fat were determined on plasma and were therefore converted to whole blood concentrations on the basis of the hematocrit values. The total amount of lactose and fat secreted in the 24-hour period was also determined. This method has the advantage over that used by Graham (9) in that excitation, which markedly affects arteriovenous differences (31), is largely avoided. If normal arteriovenous values are obtained and the total secretion of milk substances determined, it is not necessary to attempt to measure the rate of blood flow. It has a further advantage over the previous work of Shaw and Petersen in that the milk was analyzed in each experiment instead of taking the average analysis of the herd-milk. Ratios calculated on the basis of calcium and phosphorus utilization and secretion indicate that sufficient blood fat is taken up by the gland to account for 92.8 per cent of the total fat secreted. The per cent of milk fat accounted for by blood fat on this basis ranged from 84.6 to 107.9 for the five experiments.

Smith and Dastur (35) found an inverse relationship between the oleic acid and the lower acids in experiments with fasted cows, and suggested that the lower acids are formed as by-products in the synthesis of oleic acid from carbohydrate. Reinecke *et al.* (21), finding that the respiratory quotient of the mammary gland of the fasted goat was less than unity, suggested that only the lower acids were synthesized from carbohydrate. However, the fact that the R. Q. of the gland of the normal animal is greater than one, and that of the fasted animal less than one does not in any way contradict the suggestion of Smith and Dastur that the lower acids are by-products of the synthesis of oleic acid from carbohydrate. This is especially true as the synthesis of the lower acids only, from carbohydrate, would not explain the apparent inverse relationship between the lower acids and oleic acid. However, from the data presented in table 3 and on the basis of previous work it is apparent that sufficient blood fat is utilized by the mammary gland to account for most of the milk fat. It therefore appears highly improbable that both oleic acid and the lower acids of milk fat are synthesized from carbohydrate. It is true that a small quantity of oleic acid may be synthesized in this fashion, in which case the lower acids scarcely can be considered as by-products. This objection may be obviated to some extent if fat other than β-hydroxybutyric acid is oxidized for energy purposes, as has been suggested previously (24).

Hilditch (12) has emphasized that any explanation of milk fat formation must be able to account for the presence of unsaturated acids other than oleic acid in which the ethylenic linkage is situated in the same position as in oleic acid. He has also insisted that the explanation must account for "the very specific and characteristic relationships of fully saturated glyceride content to total unsaturation." He has suggested that the short chain fatty acids arise from the oxidation and subsequent reduction of oleo-glycerides. Hilditch and his colleagues have also suggested that "the final mixture of component glycerides is the consequence of a bio-hydrogenation process which has operated after the precursor fatty acids (mainly palmitic and oleic) have been assembled into triglycerides." The data of Shaw and Petersen (31) favor the proposal that the lower acids are the result of the oxidation and subsequent reduction of the longer chain fatty acids and they have suggested that this oxidation takes place to supply energy for the active mammary gland. In opposition to this suggestion is the finding of Graham (10) and others that the respiratory quotient exceeds unity. However, the respiratory quotient may be misleading and cannot in itself be accepted as conclusive evidence of the type of metabolism taking place in any one organ (36). However, it is difficult to reconcile the suggestion that the lower acids are derived from the oleo-glycerides with the findings (table 1) that there is a simultaneous increase in both the lower acids and in unsaturation following the administration of glucose to cows with ketosis. It is possible that the lower fatty acids and a small proportion of the oleic acid may be synthesized from carbohydrate. Possibly the increase in unsaturation following glucose therapy was due to the formation of lower unsaturated fatty acids as well as oleic acid. It is significant that butyric and caproic acids, as shown by the Reichert-Meissl values, account for most of the increase in the lower acids following glucose therapy. The caprylic and capric acids, as shown by the Polenske values, did not increase following glucose administration and in some cases did not return to normal for several weeks after the butyric and caproic acids had regained the normal level.

Considerable work has been done on the utilization of carbohydrate by the lactating mammary gland, and previous publications (9, 26) indicated that practically all of the available glucose and lactic acid taken up by the mammary gland is required for lactose synthesis, leaving little available capacity for fat synthesis or for energy purposes. A small additional uptake of carbohydrate in the form of glycoprotein has been reported (22). We have found in recent studies (unpublished) that the active gland uses very little lactic acid, contrary to earlier reports (9, 26). Of even greater significance is our recent work (34) in which we were unable to demonstrate any material decrease in the utilization of glucose by the gland of the cow suffering from severe hypoglycemia in ketosis, although there was a marked decrease in the lower acids of the milk fat.

The data in table 3 indicate that the amount of glucose taken up by the mammary gland may be slightly in excess of the amount required for lactose synthesis and suggest that a small quantity may be available for fat synthesis or for energy purposes. While such calculations undoubtedly involve some error, they do show that most of the milk fat and milk sugar are probably derived from blood fat and blood glucose respectively.

As the body obtains all of its glucose from protein and the glycerol portion of fat after the first few days of fasting, blood glucose continues to be available to the mammary gland and the level of blood glucose does not decline readily to the low levels observed in severe ketosis. The possibility must be considered, therefore, that in fasting and in ketosis substances present in the liver which may be the precursors of the short chain fatty acids of milk fat may be depleted by their conversion into glucose for use by the mammary gland and other tissues. Likewise, although the administration of glucose in ketosis resulted in an increase in the volatile short chain fatty acids of milk fat, it is possible that the precursors of these fatty acids were preformed in the liver or even in the rumen.

In addition, a careful analysis of the data in table 1 indicates that other factors may be involved. The milk fat constants of animal N–34 on 4–19–41 were normal in every respect, yet the animal exhibited considerable hypoglycemia. A somewhat similar picture may be observed in this animal on 3–11–41 with a blood glucose value of only 25.0 mg. per cent. Likewise the milk fat constants for animal W–1 on 4–19–41 had returned to normal although the blood glucose value was only 33.0 mg. per cent. In all these cases the animals, although exhibiting hypoglycemia, were consuming feed quite normally. It will also be noted that the small but significant increase in the short chain fatty acids following the administration of glucose to animal Pa–1 occurred within the first 36 hours and that no further increase occurred although the blood glucose was higher on the following day. It may well be that rumen digestion plays a more important role in the synthesis of milk fat than we have been led to believe. Complete fasting for as little as two or three days produces a much greater decline in the short chain fatty acids than we have observed in ketosis, yet the level of blood glucose is much lower in severe ketosis. Perhaps milk fat precursors other than those generally given consideration have their origin in the rumen. In addition consideration must be given to the possible importance of the various vitamins synthesized in the rumen, some of which have been shown to be necessary for the synthesis of fat from carbohydrate (17, 18).

SUMMARY

1. In severe ketosis, characterized by ketonemia, hypoglycemia, and varying degrees of inanition, the short chain fatty acids of milk fat were considerably below normal. Consequently β-hydroxybutyric acid cannot be considered the precursor of these fatty acids.

2. On the basis of the present evidence it appears that β-hydroxybutyric acid is one of the chief sources of energy for the active mammary gland.

3. The administration of large quantities of glucose to cows with ketosis was followed by a significant increase in the water-soluble steam-volatile fatty acids of milk fat within 36 to 48 hours. This was accompanied by an

increase in the unsaturation of the fatty acids. The water-insoluble steam-volatile fatty acids were not immediately affected, but increased following the recovery of the animal.

4. The short chain fatty acids of milk fat are not decreased nearly as much by ketosis as by short periods of fasting, although the blood glucose is usually lowered at least 50 per cent more by severe ketosis than by a few days of fasting.

5. The percentage of short chain fatty acids in the milk fat are much more closely associated with food intake than with the level of blood glucose, lactic acid, or acetone bodies.

6. The low R. Q. of the mammary gland of the fasted cow is probably due, in part, to the oxidation of β-hydroxybutyric acid.

7. In five experiments the total amount of blood traversing the mammary gland in a period of 24 hours was calculated from the utilization of calcium and phosphorus per 100 cc. of blood and the total amounts of calcium and phosphorus secreted in a 24-hour period. Each unit of volume of milk required 494 volumes of blood. Similar analyses indicated that the blood fat removed would account for 92.8 per cent of the milk fat secreted, and that the blood glucose removed would account for 105.5 per cent of the lactose.

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THE EFFECT OF COPPER ON LIPASE ACTIVITY IN CHEDDAR CHEESE

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A knowledge of the properties and the behavior of milk lipase is necessary in order to understand certain lipolytic changes which take place in milk and milk products. In a previous publication (3) the authors presented evidence to show that active milk lipase is in a reduced chemical state; that mild oxidation such as aeration in the presence of copper inactivates it; and that reactivation of milk lipase is possible by means of appropriate reducing systems.

Although these results were obtained from experiments on raw milk it was possible to draw certain conclusions regarding the probable behavior of milk lipase in raw milk cheddar cheese in relation to the development of rancid flavor. It was stated that the effectiveness of oxidative inhibition of milk lipase would be offset by the reducing systems in the cheese. It was, however, necessary to check our findings with the making of experimental cheese. Accordingly, the effect of copper on added pancreatic lipase in cheddar cheese is reported, confirming our previous conclusions.

In all, 10 comparison or duplicate vats of cheese (2) were made. Each pair of cheese was identical in every respect and included the same amount of pancreatic lipase added to the cheese milk. The first cheese of each set, however, contained added copper ($CuCl_2$ was used), while its mate did not. The level of pancreatic lipase used ranged from 0 to 2.5 gm. per 1000 lbs. milk. This gave both suboptimal and sufficient amounts of lipase necessary to produce a rancid flavor in the cheese. The amounts of copper were 2 and 5 parts Cu per million parts milk, levels which were known to be effective in inhibiting lipase on the basis of our previous experiments. The finished cheese were cured under the usual conditions and were graded for flavor at suitable intervals.

During the manufacture of these cheese a striking difference was noted in the flavor of the curd in the two vats, the difference being most pronounced at the milling stage. The curd in the vat which contained added copper was less rancid and generally superior in flavor to its mate to which no copper had been added. However, grading figures on the cheese after two weeks and subsequently showed no significant differences in the flavor scores. Detailed data are, therefore, not included.

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The results obtained with experimental cheese confirm those previously found with milk. The main conclusion is that copper is not an effective lipase inhibitor in cheddar cheese. That copper does inactivate added lipase follows from the suppression of rancid flavor development while the cheese is in the curd stage. That this effect is not permanent is indicated by the fact that no significant difference in the flavor scores or in the intensity of rancid flavor was found at grading. The explanation for this is to be found in the reactivation of oxidatively inactivated lipase in presence of the reducing systems of cheese. Although copper is known to raise the oxidation-reduction potential of cheese (1) this rise is apparently insufficient to alter the conditions in the cheese appreciably. The potential of the cheese still remains at a sufficiently negative value to be able to reduce any oxidized lipase to its active condition. It is therefore unlikely that slight variations of the oxidation-reduction potential or copper contamination would have any effect on the spontaneous development of rancid flavor under commercial conditions. Two other points may be noted in passing. The behavior of pancreatic lipase is very similar to that of milk lipase and, therefore, the use of the former for experimental purposes in dairy research seems justifiable. Also experiments with milk, used judiciously, are of value in the study of cheese. It is understood, of course, that such experimental expedients as these must ultimately be confirmed on the basis of cheese manufacture.

SUMMARY

Although copper inhibits lipase activity in milk under ordinary conditions it is ineffective as a lipase inhibitor in cheddar cheese. This is attributed to the strongly reducing potential of the cheese.

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THE UTILIZATION OF UREA BY RUMINANTS AS INFLUENCED BY THE PRESENCE OF STARCH IN THE RATION*

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In an earlier publication (3) we discussed the influence of the level of protein in the ration on the utilization of urea by a heifer with a rumen fistula. The data indicated that when the protein level in the concentrate mixture was above 18 per cent, any added urea was poorly converted into protein. The concentrate mixture used in these experiments consisted of natural grains with a high content of starch. Apparently at high levels of protein intake, even in the presence of a fermentable carbohydrate, the proteins themselves share or become prominent sources of nitrogen for the developing flora of the rumen. This information is of practical importance and would govern the level of protein and urea most suitable for maximum urea utilization in any feed formula.

Another question of equal importance in relation to the problem of urea utilization is one centering around the presence of carbohydrate in the ration. Would it be possible to meet the nitrogen requirement of a polygastric animal receiving timothy or similar hay only by the addition to the ration of urea? This is assuming that timothy (or alfalfa) hay has little readily fermentable carbohydrate in the sense that its carbohydrate content could be changed to a soluble sugar which could then serve as a ready source of energy for a developing flora. Would a material like sugar cane residue, sugar beet pulp, or orange pulp fortified with urea be an efficient nitrogenous ration for a dairy cow? These questions must be answered before a general application of urea feeding can be put into practice. It will be necessary to investigate the influence of the kind and quantity of carbohydrate in the ration on the most efficient utilization of urea if wastefulness of urea nitrogen is to be avoided. It is apparent from data (2) secured in the feeding of milch cows, as well as through rumen studies, that urea is well utilized when added to a grain mixture of corn and oats with their abundant supply of starch. Diastatic action of microbiological origin in the rumen is rapid. Further, the addition of corn molasses to a grain ration did not increase the efficiency of urea utilization.

EXPERIMENTAL

Our first experiments were concerned with the influence of starch on the utilization of urea. For this study we used a 1,000 pound Holstein heifer

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with a rumen fistula equipped with a removable rubber plug to facilitate sampling. The animal was fed twice daily, at 8 A.M. and 8 P.M. The plan of the experiment was as follows:

Period 1—Timothy hay only.

The hay was run through a hammer mill. Twelve pounds per day were allowed. The animal received this ration for a period of 3 weeks before sampling was begun. After 3 weeks as an adjustment period samples of rumen content were taken twice a week (Friday and Sunday) for a period sufficiently long to insure constancy in the determinations. Samples were taken at intervals of 1, 3, and 6 hours after feeding. About a kilo of material was taken, of which 400 grams were used for the dry matter determination and 20 gram triplicate samples of the wet material for urea, ammonia, and total nitrogen determinations. The urea was determined by the urease method and the ammonia by magnesium oxide distillation. Ammonia-N and protein were calculated as per cent of the total dry matter present. Protein was calculated as (total N minus $NH_3 - N$) × 6.25. In succeeding periods the procedure was the same as in Period 1; between periods, 3 weeks on the new ration were always allowed before chemical studies were undertaken.

Period 2—Timothy hay 10 pounds + corn starch 4 pounds daily.

Period 3—Timothy hay 10 pounds + starch 4 pounds + urea 150 grams daily.

Period 4—Timothy hay 10 pounds + urea 150 grams daily.

Period 5—Timothy hay 10 pounds + starch 4 pounds + casein 0.4 pound. This amount of casein is approximately equivalent in amount to the protein of 4 pounds daily of a mixture of corn and oats.

Period 6—Timothy hay 10 pounds + starch 4 pounds + casein 0.4 pound + urea 150 grams daily.

The data are summarized in table 1, and in figure 1 a graphic display

TABLE 1

The record of the rate of disappearance of ammonia and increase of protein in the rumen of a heifer

Ration	Ammonia-N (Per cent of dry weight)			Protein (Per cent of dry weight)		
	1 hour	3 hours	6 hours	1 hour	3 hours	6 hours
	%	%	%	%	%	%
1. Timothy hay	0.04	0.03	0.03	8.0	8.3	8.2
2. Timothy hay + starch	0.03	0.02	0.02	6.8	6.8	6.9
3. Timothy hay + starch + urea	0.19	0.13	0.04	9.5	9.7	10.7
4. Timothy hay + urea	0.15	0.23	0.11	7.8	7.7	7.5
5. Timothy hay + starch + casein	0.09	0.06	0.03	9.0	8.8	9.2
6. Timothy hay + starch + casein + urea	0.20	0.27	0.18	9.4	8.8	9.2

of the important results is presented. When timothy hay alone was fed (Period 1) both the ammonia-N and total protein were at low levels, and remained almost constant throughout the trial period. When urea was fed with the hay (Period 4) hydrolysis of the urea to ammonia was delayed, being incomplete at one hour after feeding, and disappearance of the ammonia was very slow, about half remaining as such in the paunch six hours after feeding. The protein level was slightly lower than on timothy hay alone. From these results it is evident that no abundant and active flora was operating in the rumen.

In contrast, when starch was fed in addition to the timothy hay and urea (Period 3), microbiological activity was very great. The urea was completely hydrolyzed in less than one hour, and the ammonia thus formed had practically all disappeared in six hours. As the ammonia-N level fell there was a concurrent rise in protein, indicating that the ammonia was being built into protein. The total rise in protein was approximately equivalent to the amount of ammonia disappearing from the paunch in the same period.

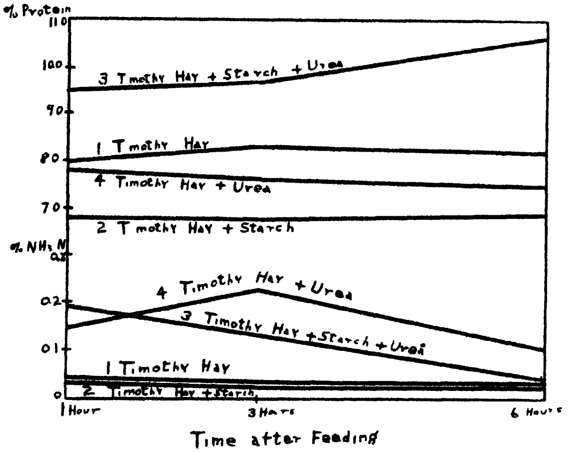

FIG. 1. Protein and ammonia-N levels of rumen contents as influenced by starch

The rapid hydrolysis of the urea, the speedy disappearance of the ammonia thus produced, and the marked rise in protein content all are evidence of a very active flora. That the flora was very abundant is indicated by the fact that the protein level when timothy hay, starch, and urea were fed was from 2.0–3.5 per cent higher than when timothy hay and urea or timothy hay and starch were fed. This difference in protein content can be due only to the bacterial proteins present, since the amount of protein supplied in the ration was the same in all cases.

The only effect of starch, when fed with timothy hay (Period 2) was to dilute the hay, lowering the protein level to below 7 per cent of the total dry matter. There was no evidence of increased microbiological activity on the rumen contents.

It was thought that the addition of small amounts of preformed amino acids (as casein) might cause more efficient utilization of the urea. However, unexpected results were obtained in periods 5 and 6. When urea was added to timothy hay, starch, and casein, the initial analyses showed efficient utilization of the urea, but as the period progressed the utilization decreased, and at the end of six weeks there was little or no utilization. Hydrolysis of the urea was complete only after three hours, most of the ammonia was still present as such in the paunch after six hours, and the protein level was the same as that found without urea. These results are difficult to explain, as the casein level of 0.4 pound per day was the same as the amount of corn and oat protein fed when a grain mixture of equal parts of corn and oats was used in addition to a daily ration of corn silage and timothy hay. On this ration there was efficient utilization of urea. It is possible that there was something about the constitution of casein that made it a preferred nitrogen source as compared with the urea. However, this would still not explain the slow rate of hydrolysis of the urea to ammonia.

It is possible that the casein caused a drastic change in the character of the flora, with a resulting change in rate of urea utilization. This influence of casein should be studied further and should be accompanied by a thorough study of the rumen flora.

DISCUSSION

It is evident that only when adequate fermentable carbohydrate (starch in this experiment) is included in the ration can urea be utilized at a maximum rate and efficiency in the rumen of the cow. Its function is undoubtedly to serve as a readily available energy source for the microorganisms, enabling them to build new protoplasm in which the nitrogen from the urea is incorporated. That very large amounts of protein can be built by the organisms from a simple organic nitrogen source like urea is shown by the fact that the addition of urea to timothy hay and starch causes a rise in the protein content of the rumen from 6.8 to 10.7 per cent, or a 57 per cent increase. Most of the urea nitrogen is built into protein; the amount of NH_3-N disappearing from the rumen contents in six hours (Period 3) is approximately equivalent to the nitrogen contained in the additional protein formed. This additional protein is undoubtedly utilized by the cow, breakdown products of the bacterial protein being absorbed either farther along in the gastro-intestinal tract, or possibly in the rumen itself. It has been shown that dextrose is rapidly absorbed directly from the rumen of the sheep (1) and it is quite possible that amino acids may also be absorbed there.

The effect of various ration constituents on the efficiency and rate of urea utilization should be thoroughly studied, so that when and if urea becomes a practical nitrogen source for the farmer, urea-containing rations can be intelligently constructed to obtain the best results with as little waste as possible.

The relation of other carbohydrates to urea utilization are being studied, and the results will be published later.

SUMMARY

1. Further studies on urea utilization in the rumen fistula heifer are reported.

2. With timothy hay as the sole ingredient of the basal ration, utilization of added urea took place only partially, if at all. In the presence of starch a suitable substrate was provided for the development of an active flora and urea was efficiently utilized.

3. When casein was added to a timothy hay-starch-urea ration the utilization of urea was markedly reduced.

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THE EFFECT OF SHARK LIVER OIL ON MILK AND BUTTER FAT PRODUCTION[1, 2]

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The detrimental effect of cod liver oil upon milk and butter fat production has been shown by Golding *et al.* (6) and others (7, 9, 11). Recently Deuel and co-workers (2, 5) reported that the feeding of shark liver oil high in vitamin A potency increased the milk and butter fat production of Guernsey cows 10 per cent or more. These workers fed 30 to 60 cc. of shark liver oil daily which was sufficient to furnish a daily intake of 700,000 to 1,400,000 I.U. of vitamin A. Practical farm supplements of shark liver oil with a vitamin A potency of 7,500 I.U. per gram have been fed to milking cows at the rate of 15–25 grams per cow per day without any noticeable effect upon either milk or butter fat production (13).

Further experiments have recently been conducted to determine the effect of shark liver oil feeding upon milk production, butter fat percentage and vitamin A in blood plasma and milk. The results of these experiments are reported herewith.

EXPERIMENTAL

The first experiment extended from February 5, 1941, to April 2, 1941, and was designed to determine if shark liver oil could be safely used as a vitamin A supplement for dairy cattle. For this purpose seven cows in the late stages of lactation were used; three Holsteins, two Guernseys, and two Brown Swiss. They were fed 25 cc. of shark liver oil[3] with a potency of either 7,500 or 15,000 I.U. of vitamin A per gram (187,000 and 375,000 I.U. of viamin A per day).

With the appearance of the report of Deuel and co-workers (2) a second experiment was conducted from May 24, 1941, to July 11, 1941, since the levels of vitamin A fed in our first experiment were not as large as those used by Deuel. Five cows just past their peak of milk production were fed 90 cc. of shark liver oil daily with a potency of 15,000 I.U. of vitamin A per gram. A second lot of five cows which were comparable in milk production and stage of lactation was used as a control lot on milk and butter fat production.

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[2] A preliminary report of a portion of these studies was presented before the Dairy section of the American Society of Animal Production, Jour. Animal Sci., 1: 68. 1942.

[3] We are indebted to Bioproducts, Inc., Astoria, Oregon, for their generous supplies of shark liver oil used in these experiments.

Unfortunately, the shark liver oil feeding was interrupted from June 16th to June 30th in this experiment, therefore, the actual feeding period covered only 48 days.

A third experiment was then outlined to check Deuel's levels of shark liver oil feeding exactly. The cows were put under preliminary observation from November 5, 1941, until December 5, 1941, then shark oil feeding was started. Three lots of cows were used. As in the previous experiments, the cows were maintained on the regular University milking herd ration and were fed the shark liver oil as later indicated. Eight cows in Lot I, which included three Holsteins, three Guernseys, and two Jerseys, were fed the herd ration without added shark oil. Lot II was composed of two Holsteins, one Jersey, and one Guernsey. They were fed the herd ration plus 50 cc. daily of shark liver oil containing 26,650 I.U. of vitamin A per gram. Thus their vitamin A intake from the oil represented 1,332,500 I.U. of vitamin A per day and closely approximated Deuel's levels of feeding. Lot III, composed of two Jerseys, two Guernseys, a Brown Swiss and a Holstein, was fed the herd ration plus 50 cc. daily of shark liver oil with a vitamin A potency of 15,400 I.U. per gram or an additional vitamin A ingestion of at least 770,000 I.U. per cow per day. Most of the cows were in the first part of their lactation period. One Jersey in Lot I and one in Lot II were eliminated before the close of the experiment because they were going dry, otherwise, the rest freshened early enough to obtain preliminary blood plasma and milk data or were fresh at the beginning of the experiment.

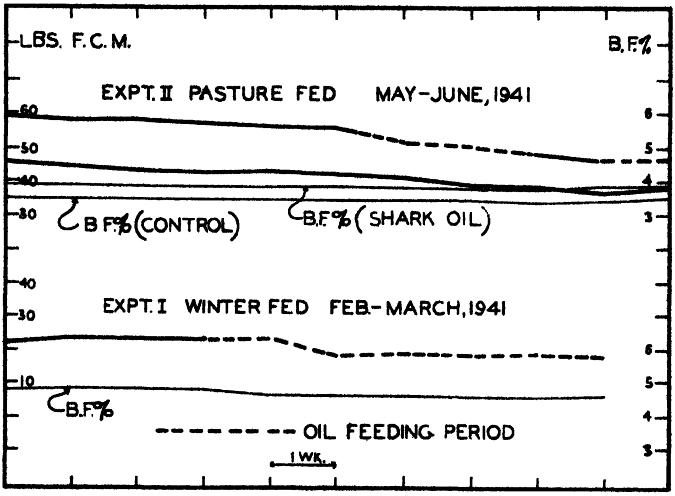

FIG. 1. Average daily milk and butterfat production.

Blood plasma vitamin A and carotene were determined by the method of Kimball (8). Ascorbic acid was determined by the Mindlin and Butler method (10). The vitamin A and carotene in the milk was determined by a modification of the procedure of Olson, Hegsted, and Peterson (11).[4]

RESULTS

It is apparent from figure 1 and table 1 that feeding 25 to 90 cc. of shark liver oil daily had no demonstrable, and particularly no stimulating effect upon the milk or butter fat production or upon the per cent of butter fat. This lack of effect was noted whether the animals were given 117,500 I.U. or as much as 1,350,000 I.U. of vitamin A per day.

TABLE 1

The effect of shark liver oil feeding upon milk and butter fat production and the vitamin A and carotene content of milk, Experiment III

Period	Lot I Basal ration				Lot II Basal + 26,650 unit oil				Lot III Basal + 15,400 unit oil			
	Ave. carotene, γ/cc.	Ave. vitamin A, γ/cc.	Ave. daily milk yield, lb. (4%)	Ave. B.F., %	Ave. carotene, γ/cc.	Ave. vitamin A, γ/cc.	Ave. daily milk yield, lb. (4%)	Ave. B.F., %	Ave. carotene, γ/cc.	Ave. vitamin A, γ/cc.	Ave. daily milk yield, lb. (4%)	Ave. B.F., %
Preliminary period												
1st wk.			26.1	5.0			31.4	4.4			24.7	4.3
2nd wk.			25.3	5.0			30.5	4.4			23.8	4.3
3rd wk.			30.8	4.3			29.7	4.4			24.9	4.3
4th wk.	0.32	0.14	34.3	4.5	0.31	0.15	32.4	4.2	0.31	0.12	24.7	4.3
Experimental period												
1st wk.	0.38	0.15	34.8	4.4	0.24	0.45	34.9	4.5	0.35	0.42	23.6	4.3
2nd wk.	0.31	0.20	34.2	4.4	0.26	0.78	34.7	4.3	0.28	0.75	23.6	4.3
3rd wk.	0.33	0.20	35.2	4.3	0.18	0.89	33.6	4.3	0.23	0.63	23.4	4.4
4th wk.			34.4	4.0			33.8	4.3			23.8	4.3
5th wk.	0.30	0.21	35.0	4.3	0.17	0.96	34.3	4.8	0.20	0.80	22.4	4.0
6th wk.	0.30	0.24	33.8	4.4	0.15	0.88	31.0	4.1	0.20	0.54	23.9	4.3
7th wk.	0.31	0.19	32.4	4.4	0.09	0.73	29.4	4.1	0.25	0.63	23.3	4.3
8th wk.	0.35	0.19	34.2	4.9	0.14	0.97	29.6	4.5	0.18	0.75	23.5	4.6

The feeding of shark liver oil brought about a distinct rise in the blood plasma vitamin A concentration as shown by table 3. It appears from these data that an equivalent level of blood plasma vitamin A was attained by feeding either the 15,400 or the 26,650 unit shark liver oil. It is apparent that the cows used in this experiment were adequately fortified with vitamin A at the beginning of the experiment since the blood plasma vitamin A was 0.19 microgram or more per cc. There was a marked decline of carotene in the blood plasma with the progress of the experiment in those lots receiving vitamin A supplements (tables 2 and 3) as compared with controls or their own original base level of carotene in the blood. The explanation of this phenomenon is not at the moment clear.

[4] Details of this procedure will be published later.

There seemed to be no beneficial effect upon the ascorbic acid content of blood plasma as the result of feeding massive doses of vitamin A to these cattle which were in good nutritive condition and whose blood plasma indicated ample vitamin A at the beginning of the experiment. This is in contrast to the increase in blood plasma vitamin C which resulted when cattle with low plasma vitamin A values were fed additional vitamin A (1).

The carotene content of the milk likewise dropped during the course of the shark liver oil feeding. The control lot in experiment III showed 0.32γ/cc. during the preliminary experimental period (table 1). This fluctuated very little or none throughout the eight weeks under experimental observation. The carotene content of the milks of lots II and III was 0.31 during the preliminary period, while at the end of the shark liver oil feeding period these values had fallen to 0.14 and 0.18γ/cc. respectively. Meanwhile the vitamin A content of the Lot I milk remained nearly constant at 0.20γ of vitamin A per cc. During the shark liver oil feeding the vitamin A content of the milks rose from 0.15 to 0.97γ/cc. and from 0.12 to 0.75γ/cc. for lots II and III, or a sixfold increase.

TABLE 2

The blood plasma carotene values

Cow No. and breed	Carotene, γ/cc.	
	1/24/41	4/2/41
Experiment I. Shark liver oil potency 7500 I.U. per gram		
123 H	2.5	1.1
844 BS	1.9	1.9
465 G	7.3	3.3
Shark liver oil potency 15,000 I.U. per gram		
90 H	2.4	0.72
131 H	2.1	1.10
467 G	5.1	3.20
851 BS	3.1	2.2
Experiment II. Shark liver oil potency 15,000 I.U. per gram		
	5/24/41	7/11/41
58 H	15.96	7.80
63 H	15.50	5.08
96 H	14.14	6.22
840 BS	10.21	6.83
1438 H	12.39	5.55
1480 H	16.46	5.60

DISCUSSION

It is obvious from these data that the feeding of high vitamin A potency shark liver oil did not increase the production of milk or butter fat or influ-

TABLE 3

The effect of shark liver oil feeding upon blood plasma vitamins A, C and carotene (Experiment III)

Period of experiment	Lot I, basal only			Lot II + 26,650 unit oil			Lot III basal + 15,400 unit oil		
	C	Carotene	A	C	Carotene	A	C	Carotene	A
	mg. %	γ/*cc.*	γ/*cc.*	*mg. %*	γ/*cc.*	γ/*cc.*	*mg. %*	γ/*cc.*	γ/*cc.*
Average base data (3 wks. prior to exp.)	0.40	5.48	0.19	0.43	5.10	0.20	0.350	5.20	0.25
13th day of exp.	0.36	4.83	0.20	0.31	3.06	0.35	0.300	3.07	0.34
27th day of exp.	0.40	4.80	0.19	0.45	2.18	0.35	0.340	2.30	0.30
48th day of exp.	0.43	4.16	0.26	0.43	1.69	0.37	0.420	1.75	0.33
53rd day of exp.	0.44	5.54	0.21	0.40	1.90	0.34	0.450	2.08	0.29

ence the per cent of butter fat present in the milk. This seemed to hold irrespective of the dose of vitamin A given or the amount of oil fed. Thus these experiments do not support the studies of Deuel *et al.* (2, 5) to the effect that shark liver oil increases milk production.

It should be pointed out that the cattle used in these studies were fed practical dairy rations and were in a good nutritive status when the studies began. When vitamin A or carotene is the limiting factor of a ration an increased milk production might be expected from the feeding of additional vitamin A. These studies, however, indicate that additional amounts of vitamin A do not stimulate milk or butter fat production in the case of well-fed dairy cows.

Our studies on milks confirm Deuel's work (3, 5), namely, that dietary measures can be used to increase the vitamin A potency of milk. However, the efficiency of increasing the vitamin A content of milk by feeding shark liver oil is very low. Calculations made from the data of these experiments show that when cows were fed 1,333,000 I.U. of vitamin A daily, an average of only 2.2 per cent of the vitamin A fed actually appeared in the milk.

The decrease in the blood plasma and milk carotene noted when shark liver oil was fed is in agreement with the recent report of Deuel and co-workers (4). In our experiments the feeding of shark liver oil resulted in a distinct rise in the level of blood plasma vitamin A. However, the level of blood plasma vitamin A, which can be obtained by the feeding of shark liver oil, appears to be limited. The cows which received 770,000 I.U. of vitamin A daily attained blood plasma vitamin A levels equivalent to the levels attained by the cows which received 1,332,500 I.U. of vitamin A daily. It is recognized that the values for the blood plasma vitamin A may be slightly inaccurate because of the difficulty in determining vitamin A in the presence of relatively large amounts of carotene (14).

SUMMARY

The effect of feeding various levels of shark liver oil of high vitamin potency to milking cows has been studied and the following results obtained.

1. Blood plasma and milk carotene of all the cows fed shark liver oil decreased during the experimental period while high amounts of vitamin A were being administered.

2. The administration of shark liver oil was effective in increasing the blood plasma vitamin A concentration. Coincident with the increased levels of blood plasma vitamin A there was a 3- to 5-fold increase in the vitamin A content of milk. These increases were effected within limits, by the vitamin concentration of the oil, the dosage given, and the length of the feeding period. Thus the vitamin A in both the blood plasma and milk rose as the result of feeding high vitamin A potency shark liver oil to cows fed practical rations.

3. Feeding shark liver oil containing high concentrations of vitamin A to cows resulted in *no increase in milk or butter fat production.* The per cent of butter fat was not affected. Stage of lactation, season, or the yield of milk up to 60 pounds per day had no favorable effect upon the reaction to shark liver oil feeding. The feeding of as much as 90 cc. of shark liver oil per cow per day tended to increase the normal rate of decline of milk production with the advance of lactation.

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FACTORS AFFECTING SWISS CHEESE STARTER ACTIVITY. EFFECT OF HEAT-TREATMENT AND SOURCE OF MILK

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The early development of a rapidly rising acidity in the curd is essential to the production of high quality Swiss cheese. The acidity, which should reach pH 5.0 to 5.15 after 21 hours (11), tends to inhibit the development of defects in the cheese: oversetting with eyes (6), wet cheese (5), and growth of gas-formers (22). In 1915, Doane and Eldredge (7) advocated the use of a pure culture of *Bacillus* (*Lactobacillus*) *bulgaricus* as a starter for Swiss cheese to prevent the occurrence of such defects. Since that time, many bacteria have been isolated from milk, whey, and Swiss cheese and have been used as pure culture starters (3, 4, 9, 10). Frazier and associates (10) in 1933 noted that few pure cultures were being used, most starters consisting of whey from a previous run, or of skimmilk inoculated with the whey. Recently, the use of pure cultures of high-temperature lactobacilli and streptococci as starters has become more prevalent.

Coincident with the widespread use of pure cultures, a problem of starter activity has arisen. It has been found in the Swiss cheese industry that occasionally a starter will fail to produce the acid fermentation in the curd with necessary rapidity. This was noted also by Elliker and Frazier (8). Indubitably, two factors are involved in the slow acid development: first, the use of milk containing large numbers of competitive microorganisms, and second, the attenuation of the biochemical activity of the starter. An investigation of the latter factor is reported here.

The experimental procedure was based on the hypothesis that two variable factors might have significant effects upon starter activity. The first of these concerns the "sterilization" of skimmilk used to propagate the starters, by exposing it to steam, flowing or under pressure. The intimate association of one of us (H.H.W.) with the Swiss cheese industry in Ohio has revealed that the heating period for skimmilk, to be used as a starter medium, varies widely among different factories and even within any one factory. Such variations (up to 5 hours) in time of heating might affect starter activity appreciably, due to alteration in the availability of nutrients or in the redox (oxidation-reduction) potential. The latter factor was considered important, since the growth of the bacteria used in pure culture starters, particularly those resembling *Lactobacillus bulgaricus*, is favored by a lowered oxygen tension, or by the presence of reducing compounds (18). In this

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connection, Hammer and Baker (13) noted that heating milk at temperatures considerably above the pasteurization temperature caused a marked increase in the rapidity of coagulation by butter starters. Stimulation of the growth of *Lactobacillus helveticus* was reported by Elliker and Frazier (8), when the culture was inoculated into reconstituted skimmilk which had been steamed 10 minutes.

The second factor to be considered was the variation in composition of milk as affected by its source. Baker and Hammer (1) reported that the source of milk had a marked influence on the acidity and flavor qualities of butter starters. The addition of lactose, milk ash, cream, or water to milk generally lowered the quality of the starter produced from it.

MATERIALS AND METHODS

Cultures. The bacterial cultures used in this study were obtained from various sources, all of them being cultures which have been used as Swiss cheese starters. Designations used were those given by the persons from whom the cultures originally came. The starters were segregated as follows, the source of each being given in parentheses:

Streptococcus cultures: *S. glycerinaceus* Orla-Jensen (Switzerland) and C3 (isolated from whey);

Streptococci with yeast[2]: MC, X, and A (whey), and C (Switzerland);

Lactobacillus cultures: *L. bulgaricus* (type culture), *L. acidophilus* and 2 (Kulp and Rettger), *Thermobacterium helveticum* (Orla-Jensen), *Thermobacterium lactis* 39E19, R–9, and R–44 (Switzerland), B2 (whey), 39aH (Hastings), and 14 (from feces of an acidophilus-fed dog);

Lactobacilli with yeast[2]: GA and G_2O (Wisconsin) and DL rods–Swiss (unknown origin).

In addition, pure cultures of the bacteria were isolated from some of the mixed cultures of bacteria and yeast. These were designated by the name of the parent culture, with a "p" added: MCp, Xp, Ap, GAp, and DLp.

As recorded by Tyler (21), the lactobacilli belong to three species: *L. bulgaricus* (Luerssen and Kühn) Holland, *L. acidophilus* (Moro) Holland, and *L. helveticus* (Orla-Jensen) Bergey *et al.* Most of them are of the last-named species. The streptococci may be classified as *S. thermophilus* Orla-Jensen, with the exception of the named species, *S. glycerinaceus* Orla-Jensen (*S. faecalis* Andrewes and Horder).

Heat-treatment of milk. Samples of milk from a single source were collected at the receiving platform and heat-treated as soon as possible. Heating at 80° C. was carried out in a thermostatically-controlled water bath, at 100° C. in flowing steam, and at 120° C. in an autoclave at 15 pounds pressure.

Redox potential determinations. The redox potential of the milk, deter-

[2] An unclassified, non-sporulating, film yeast, "Mycoderma" (20).

mined immediately before and after heat-treatment, was measured with platinum-foil electrodes, the saturated calomel half-cell, and a Coleman potentiometer. The average of duplicate determinations was used.

Determination of starter activity. The biochemical activity of the starters was determined by titration of acid produced in the milk after incubation at 37° C. in a water-jacketed, thermostatically-controlled incubator for various periods. For titration, N/10 NaOH and phenolphthalein indicator were used. The acidity is expressed in percentage, calculated as lactic acid.

Bacterial counts. Numbers of bacteria in cultures were determined by direct microscopic count. When numbers of bacteria were too great to be counted from the culture itself, serial dilutions in distilled water were prepared, and direct counts made from the dilutions. Rogers and Whittier (17) found this method gave reliable results. All determinations are recorded as individual cell counts.

EXPERIMENTAL

Heat-treatment of milk at 80° C., 100° C., and 120° C. Representative samples of milk from the mixed milk of a large herd, collected as previously described, were heat-treated by methods intended to approximate those used in Swiss cheese factories. Heating was carried out at 80° C. for 1, 2, 3, and 4 hours; at 100° C. for 1, 2, 4, and 5 hours; and at 120° C. for 15, 30, 45, and 60 minutes. Before heating, and immediately after cooling the heat-treated samples to room temperature, the redox potential of the milk was determined. One ml. of a starter culture was added to each 100 ml. sample, followed by incubation at 37° C. The milk cultures were titrated for acidity after 12 and 24 hours of incubation

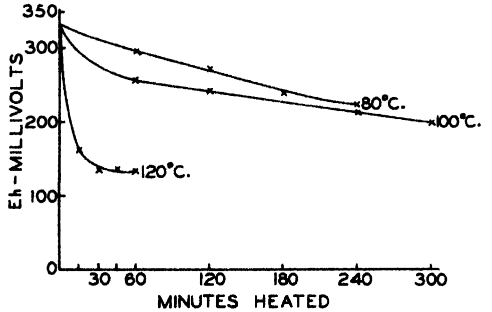

FIG. 1. Effect of heating milk at various temperatures on the potential trend. Averages of several determinations.

Figure 1 illustrates the fall in potential observed in the heat-treated milk with increase in temperature or length of heating. It may be noted that the most rapid decrease occurred during the early part of the heating period, the later decrease being a gradual, straight-line function of time. It is probable that only a part of the decrease from an initial average Eh of 330 millivolts was due to expulsion of dissolved oxygen; Gould (12) has suggested that heat-treatment of milk may release reducing groups from the milk proteins.

The biochemical activity of the bacteria was not affected significantly by heat-treatment of milk at 80° C. Heating at 100° C. for periods longer than one hour, however, had a favorable influence upon acid production by all cultures. Similar results were obtained in milk heated at 120° C. for periods longer than 15 minutes. The results of acidity determinations after 24 hours of incubation are presented in table 1. Typical results for some representative cultures, and the average of all 18 cultures used, are illustrated in figures 2 and 3. Repetition of the experiment later gave similar results.

TABLE 1

Effect of heat-treatment of milk on Swiss cheese starter activity
(Cultures incubated at 37° C. for 24 hours)

Culture	Milk heated at 100° C.				Milk heated at 120° C.			
	Hours				Minutes			
	1	2	4	5	15	30	45	60
	Percentage acidity				Percentage acidity			
S. glycerinaceus	0.48	0.51	0.53	0.50	0.50	0.50	0.56	0.60
C3	0.56	0.60	0.66	0.61	0.54	0.53	0.55	0.62
Ap	0.75	0.79	0.90	0.84	0.52	0.54	0.55	0.77
Xp	0.45	0.53	0.51	0.48	0.97	0.87	1.02	1.00
MC	0.54	0.71	0.77	0.62	1.68	1.75	1.80	1.82
C	0.47	0.53	0.73	0.64	0.84	0.90	0.93	0.94
X	0.79	0.81	0.83	0.82	0.95	0.88	1.09	1.05
Therm. lactis	0.33	0.39	0.42	0.41	0.30	0.32	0.32	0.31
Therm. helveticum	1.10	1.36	1.08	1.17	0.79	0.73	0.72	0.49
2	0.37	0.51	0.43	0.52	0.45	0.31	0.47	0.43
14	0.50	0.54	0.52	0.52	0.48	0.50	0.41	0.42
39aH	1.27	1.57	1.59	1.30	0.49	0.40	0.58	0.44
B2	2.54	3.01	2.37	2.60	1.34	1.51	1.15	1.29
R–9	1.83	1.94	1.86	1.64	2.54	2.39	2.57	2.57
DLp	0.97	1.07	1.10	0.82	0.73	0.74	0.89	1.04
GA	2.12	2.16	2.46	2.39	2.48	2.49	2.61	2.80
G_2O	1.84	2.30	1.73	1.71	2.43	2.28	2.21	2.37
DL rods	1.01	1.09	1.22	1.31	1.76	1.75	1.68	1.85

While acid production by all cultures was increased in milk heated at 100° C. for 2 to 4 hours, certain cultures were stimulated to a greater extent than others. This was true of the lactobacilli in general, and of the lactobacillus-yeast cultures particularly; such cultures were always the most active acid producers. With two exceptions, acid production of all cultures was enhanced by heat-treatment of milk at 120° C. for longer than 15 minutes. The level of activity of some of the cultures was lower in milk heated at 120° C. as compared with that heated at 100° C.; with others it was higher, while a few showed the same activity in each. The low acidities

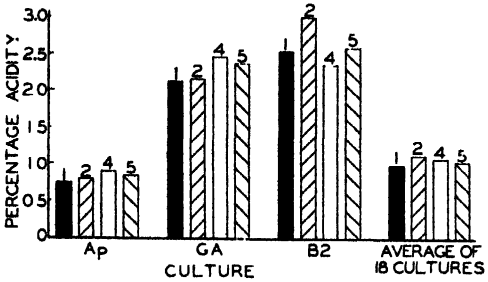

FIG. 2. Effect of heating milk at 100° C. for 1, 2, 4, and 5 hours on the acidity produced in 24 hours at 37° C. by subsequently inoculated Swiss cheese starter cultures.

produced in 24 hours by 4 of the lactobacilli (*Therm. lactis, Therm. helveticum*, 2, and 14) were typical of these cultures; apparently they had been attenuated by long artificial culturing.

Rate of growth and acid production in milk heat-treated at 100° C. In order to determine whether the rates of growth and acid production were affected by heat-treatment of milk, a similar series of experiments was carried out, using milk heat-treated at 100° C. for 1, 2, 3, and 4 hours and certain representative starter cultures. Representative samples of milk, after initial redox potential measurement, were heat-treated and the potential was again determined; the samples then were inoculated with one per cent of a starter culture. Cultures used were 39aH, B2, G_2O, *S. glycerinaceus*, Xp, and X. After incubation at 37° C. for 4, 8, 12, 24, 36, and 48 hours, aliquots were removed for direct microscopic count and determination of titratable acidity.

The results of this series confirmed the results obtained in the earlier experiments: heating milk for 2 to 4 hours resulted in increased acid production by the starters as compared with the one-hour heating. As before, the potential trend was downward with increased length of exposure to 100° C.

Representative data are presented in table 2. Interesting phenomena

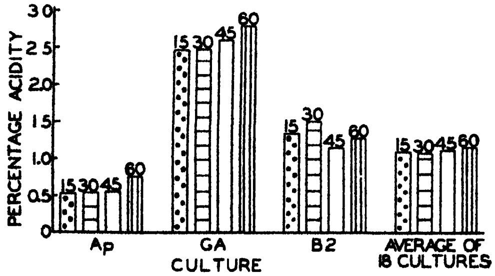

FIG. 3. Effect of heating milk at 120° C. for 15, 30, 45, and 60 minutes on the acidity produced in 24 hours at 37° C. by subsequently inoculated Swiss cheese starter cultures.

appeared when the results of direct counts and titratable acidity were plotted graphically and compared. With culture 39aH (fig. 4) the greatest growth rate during the first 24 hours of incubation occurred in milk heated for 2 or 3 hours, but there was little difference in the numbers of bacteria at the end of 24 hours of incubation or later. Growth in milk heated for one hour was slower and progressed to a lower end-point. The rate of acid production was greatest in the milk heated for 2 hours, and the acidity reached a much higher level at the end of 24 hours in such milk. Culture B2 (fig. 4) also showed a fair correlation between the rate of growth and the rate of acid production; the two-hour heat-treatment was optimal. As before, *S. glycerinaceus* (and the other streptococci) did not exhibit the striking differences in activity due to heat-treatment of the milk that were shown by the lactobacilli, but appreciable variations were observed. There was poor correlation between the rates of growth and acid production.

TABLE 2

Effect of heat-treatment of milk on the rate of growth and acid production of Swiss cheese starter cultures

(Milk heated at 100° C. Incubated at 37° C. after inoculation)

	Heated 1 hr.		Heated 2 hrs.		Heated 3 hrs.		Heated 4 hrs.	
	% acid	Count ($\times 10^7$)	% acid	Count ($\times 10^7$)	% acid	Count ($\times 10^7$)	% acid	Count ($\times 10^7$)
Heated milk...	0.15	0.0	0.16	0.0	0.19	0.0	0.19	0.0
Hours incubated	Inoculated with 1 per cent of culture 39aH							
4	0.14	0.40	0.17	0.48	0.20	0.52	0.21	0.78
8	0.17	1.0	0.21	2.6	0.22	3.0	0.23	2.7
12	0.27	4.1	0.32	1.6	0.25	0.63	0.52*	39.0*
24	0.83	5.9	1.16	51.0	0.78	17.0	0.72	54.0
36	1.35	15.0	1.46	54.0	1.29	67.0	1.32*	65.0*
48	1.67	28.0	1.47	110.0	1.48	96.0	1.59	59.0
Heated milk..	0.15	0.0	0.16	0.0	0.19	0.0	0.19	0.0
Hours incubated	Inoculated with 1 per cent of culture B2							
4	0.22	2.3	0.20	1.6	0.20	0.07	0.23	0.16
8	0.39	13.0	0.46	43.0	0.21	0.17	0.27	0.68
12	0.72	11.0	0.95	75.0	0.23	1.7	1.25*	200.0*
24	1.80	57.0	2.07	140.0	0.68	110.0	1.69	280.0
36	2.19	81.0	2.46	120.0	1.92	410.0	1.98*	480.0*
48	2.59	130.0	2.50	260.0	2.22	290.0	2.49	610.0

* These determinations were made after 18 and 32 hours, respectively.

The data obtained were utilized to calculate the fermenting capacity of

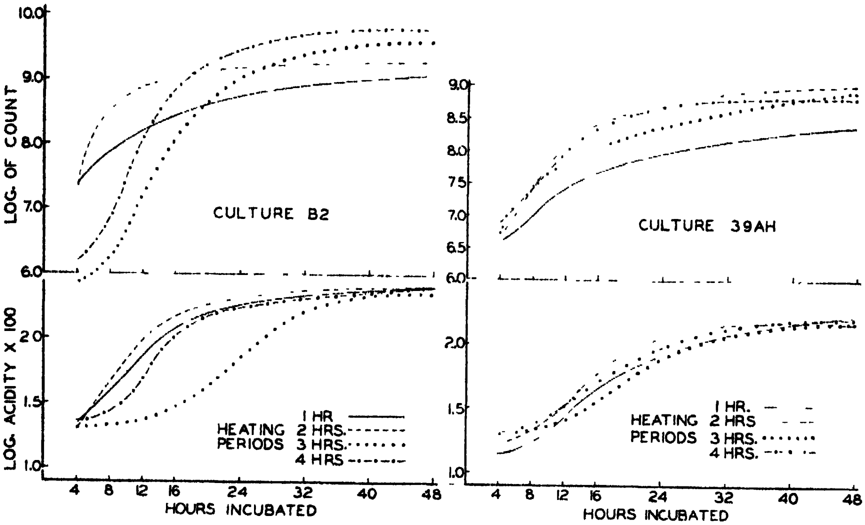

Fig. 4. Effect of heating milk at 100° C. on the rate of growth and acid production of subsequently inoculated Swiss cheese starter cultures.

the cells in the several samples, in an attempt to discover whether the increased activity was due to stimulation of cellular activity or to an increase in numbers of cells. The formula used was that of Rahn (16). The results (table 3) showed that the fermenting capacity of the cells was in general increasingly lowered in milk heated at 100° C. for the longer periods. The explanation for the increased activity must lie, then, in increases in numbers of cells sufficient to overcome the decreases in cellular fermentative power.

Effect of source of milk on activity of the starters. Random observations made during preliminary work, as well as reports published by other workers (1), indicated that the composition of the milk might affect the activity of the starters. For this reason, the source of milk for the preceding experiments was kept as constant as possible. In a study of the

TABLE 3

Effect of heat-treatment of milk on the fermenting capacity of Swiss cheese starter bacteria

Culture	Mg. acid × 10^{10} produced per cell per hour in milk heated at 100° C.			
	1 hour	2 hours	3 hours	4 hours
39aH	5.34	1.47	1.59	2.33
B2	5.25	1.04	1.31	0.70
G_2O	1.33	0.74	0.33	0.97
S. glycerinaceus	1.53	0.63	0.53	0.26
Xp	0.87	0.60	0.28	0.49
X	0.57	1.52	0.72	0.33

effect of source of milk, samples were obtained from the milk of various individual producers, all of them delivering relatively small volumes. The milk was handled as in the preceding series, the samples being heat-treated at 100° C. for 1, 2, 3, and 4 hours before inoculation. Potential determinations were made before and after heating and the titratable acidity was determined after 12 and 24 hours incubation at 37° C.

The data are too voluminous to be presented *in toto;* representative results for two cultures in a few of the milk samples are illustrated in figure 5. Again, the stimulating effect of heat-treatment was observed. Some of the differences noted in the activity in samples from different producer-sources were striking. The relative activity of all cultures was the same in any sample. It was noted that no correlation could be observed between the average Eh of the different samples and the activity of the starters; in any one sample-source, however, the potential trend was downward as the length of heating increased.

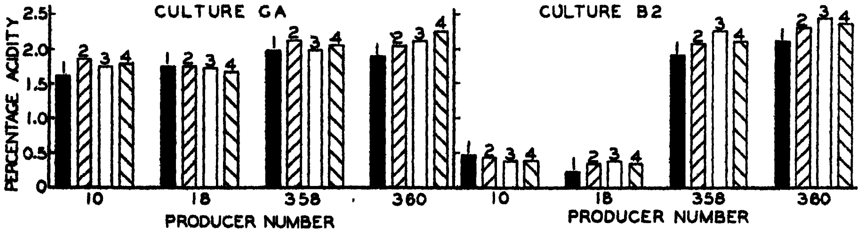

FIG. 5. Effect of the source of milk heated at 100° C. on the acidity produced in 24 hours at 37° C. by Swiss cheese starter cultures. (Figures above the columns refer to the length of heating periods in hours.)

DISCUSSION

It appears that several factors may be involved in the variations which occur in the activity of Swiss cheese starters. At least one of these factors is associated with the heat-treatment to which milk is subjected prior to inoculation with a starter. Such heat-treatment, in practice, is limited to the mother-culture medium; it is reasonable to suggest that the activity of the mother culture should be maintained at a high level. From the standpoint of elimination of competitive microbial activity, heating milk for one hour at 100° C. is usually sufficient. However, it appears that there is some justification for the use of heating periods of 2 to 4 hours. In view of the observed decrease in potential with increased exposure to the various temperatures, it is probable that the longer heating periods provided a more suitable reducing environment for the starter bacteria.

A complementary problem which warrants investigation is the effect on starter activity of serial subculturing in milk samples heat-treated under various conditions.

The composition of the milk in which Swiss cheese starters are cultured is apparently of greater importance in affecting their activity. It was observed that great differences in rate and extent of acid production occurred when milk from different sources was used. There are factors causing appreciable variations in the composition of skimmilk, such as feed, breed of cow, season, etc. The percentage variations of the major constituents (lactose, protein, ash, water) are relatively small, however, and it seems unlikely that such small differences would effect the large variations observed in starter activity. Baker and Hammer (1) found this true in the case of butter cultures, and unpublished work by us has confirmed it for Swiss cheese starters. It is suggested, therefore, that the known variations in milk of various bacterial growth factors (2, 14, 15, 19) may be the important factors in limiting Swiss cheese starter activity. A study of the effect of such variations is being carried out at present.

SUMMARY

1. Heat-treatment of milk from a single source at 80° C. for periods up to 4 hours had little effect on the activity of Swiss cheese starters subsequently inoculated.

2. Heat-treatment at 100° C. for 2 to 4 hours increased the rate of acid production by the starters over that occurring in milk heated a shorter or a longer period. Similar stimulation was observed in milk heated at 120° C. for 30 to 45 minutes.

3. The redox potential of the milk samples showed a downward trend with increase in temperature or with the longer holding periods at any one temperature.

4. The increased rate of acid production in milk heated at 100° C. for 2 to 4 hours appeared to be due to an increased growth rate, since the fermenting capacity of the cells decreased progressively in milk heated for the longer periods.

5. The composition of the milk, as determined by source, had a greater effect on the activity of the starters than heat-treatment.

6. It is suggested that important differences in the activity of Swiss cheese starters may be due to variations in (1) the oxidation-reduction potential in milk and (2) the growth factor content of milk from different sources.

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THE DENSITY OF MILK FAT: ITS RELATION TO THE ACCURACY OF THE BABCOCK TEST

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This is the first of a series of papers dealing with the factors that affect the accuracy of the Babcock fat test. The Vermont Agricultural Experiment Station in cooperation with the sub-committee on testing milk and cream of the American Dairy Science Association undertook a comprehensive regional and seasonal study of this problem.

The present paper deals with the error introduced by the deviation of density of the materials comprising the "fat" column from the assumed value of 0.9 at 60° C. This assumption has not been subjected to critical and detailed research for milk although a limited amount of data has been published on the cream test. Comparative data are given in the present study on the density and the coefficient of expansion of the Babcock column materials and purified fat from identical sources. Subsequent papers will report studies on the problem of temperature of reading, the composition of the fat column, and on the residual fat in the acid hydrolysate.

REVIEW OF THE LITERATURE

Density

Examination of the literature revealed a number of values for the relative mass of milk fat (1, 5, 6, 7, 8, 11, 12, 14, 17, 18, 19, 20, 21, 24, 25, 26, 27, 28, 29, 30, 32, 33, 34, 35, 36, 37, 38, 39, 40, 42, 43).[8] As early as 1883, Lieber-

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[6] Member of above committee, Illinois Agricultural Experiment Station.

[7] Published with permission of the Director of the Vermont Agricultural Experiment Station.

[8] This literature is reviewed in a thesis presented by Robert Jenness to the Committee on Degrees of the University of Vermont and State Agricultural College in partial fulfillment of the requirements for the degree of Master of Science, June, 1940. This thesis is on file in the library of the University of Vermont.

mann (21) emphasized the dependence of accuracy in volumetric fat tests on density values accurately determined at the temperature of reading.

Very few data were found for the resultant density of the materials estimated as fat in the Babcock test. Babcock, himself (3), *assumed* the specific gravity of these materials to be 0.9 at about 49°[9] and reported that reading the tests of milk containing five per cent fat at 43° or at 66° resulted in differences of less than 0.1 per cent. Hunziker *et al.* (15) assumed a density of 0.9 at 57°.

Recently Miller *et al.* (22) have measured the density of filtered milk fat and fat obtained from cream test columns. They report mean values at 60° of 0.89169 for filtered fat, 0.89347 for fat extracted by the Mojonnier method,[10] and 0.89512 for fat from the Babcock test. It is assumed that these values represent true density although no dimensions are given.

Two workers have attempted to account for part of the error of the Babcock test on the basis of deviation of the density of the fat from a value of 0.9 at the temperature of reading. Their calculations are based on the density of purified fat rather than on that of fat from the Babcock column. Bailey (4) reported a density value for purified milk fat of 0.8974 at 49°. This varied by 0.00068 per degree; on this basis he calculated that the density would be 0.9 at 45° and that reading the Babcock test at 54° would introduce an error of 0.032 per cent for milk averaging 4.51 per cent fat. Dahlberg (10) estimated the density of milk fat as 0.8943 at 55.5° and calculated that Babcock readings would be 0.02 to 0.03 per cent too high if read at 57.2°.

Coefficient of Expansion

Calculations of density of fat at temperatures other than those of measurement are possible if either the coefficient of expansion or the coefficient of density change is known. The literature revealed no determinations of the coefficient of expansion of fat from the Babcock test, but a few determinations and calculations for purified fat were found (21, 24, 26, 29, 39, 41). Webster (39) used such a value (0.00064) to calculate that a change of 0.0178 per cent occurs in milk testing 5 per cent fat for every 5.55° change in temperature. Cochran (9) stated that no differences in volume of fat could be observed with small differences in temperature.

Bailey's (4) value for density change of 0.00068 per degree represents a coefficient of expansion of 0.000757 based on his value of 0.8974 for density at 48.8°.

METHODS

This study was based on the fat obtained from the Ayrshire, Holstein, Guernsey and Jersey breeds of dairy cattle. Sufficient milk was obtained from each breed to nullify the effects of stage of lactation and of individuality of the cows. At the Vermont Station samples were taken weekly for one year from the Jersey and Holstein breeds, from a University composite and from a local creamery composite. At the Kansas Station samples were taken from each of the four breeds twice monthly. At Ohio State University samples were taken from each breed during February, March, April and May, while at the Illinois Station samples were taken during the same period, with the exception of May. The herds in all instances were fed and managed according to well established practices.

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[9] All temperatures are in degrees Centigrade.

[10] Mechanized modification of the Röese-Gottlieb method.

The Babcock test was conducted essentially according to official methods (2). The milk was sampled at approximately 21°, and the pipettes were filled slowly to avoid incorporation of air, and drained for approximately ten seconds, with the last milk being blown out. Sulfuric acid was tempered to 21.1°, 17.5 ml. being used of specific gravity 1.82 and 15 ml. of specific gravity 1.83. No test bottles were used that showed any inaccuracies that could be detected by the mercury method of calibration. At the Vermont Station the test bottles were shaken five minutes after the addition of acid with a shaking device sold by the Cherry-Burrell Corporation of Chicago, Illinois. At the other cooperating stations the bottles were shaken by hand. Electrically heated centrifuges were used. At the Vermont Station the tests were estimated with the aid of a special reading device (16), while at the other stations the conventional hand calipers were used.

For the specific gravity determinations the fatty materials from 24–48 test bottles were collected by suction into a 125-ml. Erlenmeyer flask directly from the water bath at 60°. The flasks were tightly stoppered and kept in a refrigerator at 7°–9°. The last four tests of each sample were left in the bath and estimated for fat. The purified milk fat was prepared from the same milk by separating 20 quarts at 26°–28°, cooling the cream to 10°–13° and churning it in a small four-quart glass churn. The butter was washed twice with water at about 10° and a portion melted in flowing steam. Babcock cream test bottles were filled to the base of the neck with melted fat and whirled in a hot centrifuge for five minutes. The clear fat was decanted into 250-ml. Erlenmeyer flasks and dissolved in approximately equal volume of reagent acetone and filtered through 15 cm. No. 1 filter paper into aluminum dishes.[11] The acetone was evaporated and the fat was dried in an oven at 23–25 inches of vacuum for 5–6 minutes and cooled in a desiccator for 5 minutes. The Mojonnier apparatus (23) was used to evaporate the acetone and to dry the fat. The samples of milk fat were kept in tightly stoppered bottles in a refrigerator at 4°–5° until the physical and chemical constants were determined. This was done within 72 hours.

The method used for determining the specific gravity was essentially that described by Siebel and Kott (31). Ten-ml. conical pycnometers with thermometers ground to serve as stoppers and with capillary overflow tubes were used. To reduce the effect of buoyancy in weighing, a counter-poise was constructed that had nearly the same displacement as the pycnometer and weighed about one gram less than the pycnometer filled with fat. The pycnometers were carefully cleaned and dried after each determination.

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[11] This step was probably unnecessary, but since it had been employed at the beginning of this work, it was continued to insure a uniform treatment to all samples.

The samples of purified milk fat were transferred to the pycnometer at about 30° with 10-ml. Mohr pipettes. Next the thermometer was inserted and the instrument placed in a water bath at 37.5° ± 0.1 for 15 minutes to permit the excess fat to escape through the capillary tube. The tube was capped and the pycnometer carefully dried with a clean cloth or with ether and cooled to room temperature of approximately 21°–22° in a desiccator and weighed. The determinations of the fatty materials from the Babcock test were made in the same manner except they were evacuated at 23–25 inches for three minutes to remove bubbles of air. All determinations were made at 37.5°/37.5° with water as the base.

To determine the coefficient of expansion, 10-ml. dilatometers with necks containing 0.5 ml. and graduated in 0.01-ml. divisions and fitted with stopcocks were used. They were calibrated with mercury at 20°–21°. In making determinations, the melted fat at about 30° was drawn in until it entered the graduated neck. The stopcock was then closed. The dilatometer was immersed in the water bath at 30° for 10 minutes and the position of the meniscus on the graduated scale recorded. The bath was heated to 40° and the reading taken again after 10 minutes. Similar readings were taken at 50° and 60°. The coefficient of expansion was calculated by the following general formula:

$$C = \frac{V_2 - V_1}{V_1(t_2 - t_1)}$$

Where:

C = expansion coefficient (in ml. per ml. per °C.).
V_1 = initial volume.
V_2 = final volume.
t_1 = initial temperature.
t_2 = final temperature.

Some check measurements were made later with dilatometers having 0.3-ml. necks graduated in 0.001-ml. divisions. The body of these dilatometers was fitted with *copper-constantan thermocouples* so as to obtain the temperature of the fat within 0.3° at any time during the determination. A Leeds and Northrup type potentiometer was used.

RESULTS

The coefficient of expansion of purified milk fat and of the corresponding materials extracted as fat in the Babcock test were determined weekly during the first six months of 1939 from the Holstein and the Jersey breeds, from composites of the University herd milk and from composites of a local milk supply. A detailed analysis of the data failed to show any relation between the values and the breed or season that might influence the Babcock test. The results for each temperature interval are summarized in table 1.

TABLE 1

*Coefficient of expansion of purified milk fat and of the materials extracted as fat in the Babcock test**

	Babcock test fat			Purified milk fat		
	°C.			°C.		
	30–40	40–50	50–60	30–40	40–50	50–60
Jersey	75.85	75.62	76.65	78.53	79.00	78.93
Holstein	75.01	75.42	74.22	78.44	78.45	78.02
Univ. comp.	75.06	75.98	75.12	78.36	78.07	79.08
Creamery comp.	75.30	76.02	76.77	78.35	78.26	76.61
Aver.	75.30	75.76	75.69	78.42	78.44	78.16
Aver. of all		75.58			78.34	

* Values to be multiplied by 10^{-5}. Coefficient of expansion is expressed in ml./ml./°C.

It is evident that the figures do not vary greatly within the temperature ranges studied. Probably due to impurities, the coefficient of expansion of the materials extracted as fat from the Babcock test was slightly lower in every temperature range than that of the milk fat. This work was checked with the more precise dilatometer on purified milk fat. The averaged results of 10 determinations for each temperature interval are summarized in table 2. These results confirm those in table 1 for purified milk fat.

TABLE 2

*Coefficient of expansion determinations of purified milk fat made with the more precise dilatometer**

Temperature	Coefficient of expansion
°C.	
30–35	76.12
35–40	77.90
40–45	81.04
45–50	80.94
50–55	78.79
55–60	79.38
Aver.	79.03

* Values to be multiplied by 10^{-5}. Coefficient of expansion is expressed in ml./ml./°C.

Effect of season. A comparison of the average density values at 60° for purified milk fat from the Holstein, Jersey and the mixed breeds over a period of one year revealed no significant monthly or seasonal differences as shown in table 3. These data show a remarkable degree of uniformity for the different breeds in two widely separated sections of the United States.

TABLE 3

Average density of purified milk fat by months during 1939

Month	Density of purified milk fat 60° in gms./ml.						
	Kansas*			Vermont†			
	Source			Source			
	Holstein and Ayrshire	Jersey and Guernsey	Mixed	Holstein	Jersey	Mixed composite	
						University	Creamery
January	0.8888	0.8889	0.8887	0.8894	0.8907	0.8902	0.8901
February	0.8893	0.8894	0.8884	0.8906	0.8907	0.8909	0.8901
March	0.8880	0.8884	0.8887	0.8896	0.8895	0.8898	0.8893
April	0.8878	0.8890	0.8886	0.8886	0.8889	0.8893	0.8891
May	0.8867	0.8875	0.8873	0.8889	0.8879	0.8879	0.8875
June	0.8894	0.8891	0.8894	0.8888	0.8900	0.8896	0.8890
July	0.8896	0.8896	0.8896	0.8881	0.8885	0.8887	0.8877
August	0.8897	0.8899	0.8895	0.8888	0.8878	0.8888	0.8886
September	0.8897	0.8894	0.8888	0.8881	0.8884	0.8884	0.8886
October	0.8897	0.8894	0.8898	0.8872	0.8873	0.8879	0.8870
November	0.8899	0.8899	0.8899	0.8880	0.8882	0.8891	0.8882
December	0.8901	0.8897	0.8900	0.8901	0.8888	0.8898	0.8889
Average	0.8891	0.8892	0.8891	0.8888	0.8889	0.8892	0.8887

* Each value represents the average of two separate determinations made during each month at intervals of approximately two weeks.

† Each value represents the average of four separate determinations during each month at intervals of one week.

These determinations were made as specific gravity at 37.5°/37.5°, but were converted to density at 60° because the materials estimated as fat are alleged to have a density of 0.9 at this temperature. The following formula was used for the conversion:

$$\text{Sp. Gr. at } 60^\circ/37.5^\circ = \frac{\text{Sp. Gr. at } 37.5^\circ/37.5^\circ}{1 + [78.34 \times 10^{-5}\ (60^\circ - 37.5^\circ)]}$$

$$\text{Density at } 60^\circ = \text{Sp. Gr. at } 60^\circ/4^\circ = \text{Sp. Gr. } 60^\circ/37.5^\circ \times 0.99318.$$

The figure 0.99318 is the density of water at 37.5°, while the factor 78.34×10^{-5} is the coefficient of expansion of the purified milk fat.

The averaged results that are summarized in table 4 likewise show no marked variations between different geographical locations.

TABLE 4

Average density of purified milk fat by breeds

Station	No. of samples per breed	Density at 60° in gms./ml.				
		Ayrshire	Holstein	Guernsey	Jersey	Mixed
California	1					0.8887
Illinois	2	0.8904	0.8905	0.8906	0.8903	0.8895
Kansas	23	0.8891*	0.8891*	0.8892†	0.8892†	0.8891
Ohio	8	0.8904	0.8905	0.8909	0.8905	0.8904
Vermont	48		0.8888		0.8889	0.8889‡

* Composite sample of milk from the Holstein and Ayrshire breeds used.
† Composite sample of milk from the Guernsey and Jersey breeds used.
‡ Average of 96 samples.

Effect of feed. Limited data have been obtained on the effect of feed on the density of milk fat. At Ohio a comparison was made of the effect of dry feed and of pasture on density. The results in table 5 show a slightly greater density for fat from three of the five groups of cows on pasture as compared with dry feeding; however, the differences are small. At the Illinois Station a marked effect on the composition of butterfat obtained by adding corn oil to a standard ration resulted in density values of 0.8892 and

TABLE 5

Relation of feed to the density of purified milk fat obtained at the Ohio station

Breed	Rations	
	Winter	Pasture
	Density at 60° in gms./ml.	
Ayrshire	0.8902	0.8908
Holstein	0.8905	0.8905
Guernsey	0.8910	0.8906
Jersey	0.8905	0.8910
Mixed	0.8902	0.8908
Average	0.8905	0.8907

0.8903, respectively, the corresponding iodine numbers of the milk fat being 51 and 44. These data, together with those in table 3 indicate that changes in the ration, season of the year or a combination of these two factors exert no significant effect on the density of purified milk fat that might cause direct variations in estimating fat by the Babcock method.

Effect of method of extraction. The specific gravity of the fatty material from the Babcock test was determined at 37.5°/37.5° and converted to density at 60° by means of the formula used for the purified fat, except that the value 75.58×10^{-5} was used as the coefficient of expansion. As shown in table 6 the fatty material showed a higher density than the purified fat.

TABLE 6

Comparison of the density of the material estimated as fat by the Babcock method with purified milk fat from the same milk

Source	Number	Average density at 60° in gms./ml.			
		Babcock test fat		Purified milk fat	
		Milk	Cream	Milk	Cream
Kansas	50	0.8915	0.8931	0.8892	0.8894
Ohio	40	0.8918	......	0.8906	
Vermont	80	0.8921	0.8921	0.8881	0.8882
Average		0.8918	0.8926	0.8893	0.8888

The mean density of the material from the milk test was 0.8918 at 60° as compared with 0.8892 for the corresponding purified fat, while the mean densities of fatty materials from the cream tests and their comparable purified fats were 0.8926 and 0.8888, respectively. The higher density of the fatty materials is undoubtedly due to the inclusion of impurities. This was shown at the Illinois Station where the fatty materials from the Babcock test and the neutral fat from the same source were purified by the method already described and both had an average density of 0.8900 at 60°.

Error in reading the Babcock test at 60° C. The Babcock test bottle is calibrated on the assumption that the fatty material has a density 0.9 at 56°–60° C. At the Vermont Station the various milks were estimated for fat as part of the routine in making the density determinations of the fatty materials. The average fat estimations of milk from the Holstein and Jersey breeds and the average densities of the fatty materials from the Babcock test for the year 1939 are summarized in table 7, together with the calculated errors and corrected readings. The following formula was used to compute the error:

$$\text{Error} = \text{Babcock reading at } 60° - \left(\text{Babcock reading} \times \frac{\text{Density at } 60°}{0.9}\right)$$

The materials estimated as fat in milk by the Babcock method have a density of less than 0.9. The calculations indicate an overreading of as much as 0.05 per cent on milk of high fat content, while the difference is not so great on milk of lower fat content. In these calculations it is assumed that the fatty materials reach an equilibrium temperature of 56°–60° within five minutes after the test bottles are placed in the water bath, and that the entire fat column consists of fatty materials—although it includes the upper meniscus and the lower part which is usually curved and not horizontal.

The fatty materials from the Babcock test showed greater fluctuations in density than did the purified fat. This is evident by comparing the density of the purified fat of the Holstein and Jersey breeds from the Vermont Station in table 3 with the density of the corresponding fatty materials from the Babcock test of the same breeds in table 7. There was a tendency for the densities of fatty materials estimated by the Babcock test to show a gradual decrease beginning in early summer and continuing until fate fall, especially for the Jersey fat; this was not so evident for Holstein fat.

TABLE 7

Error in reading the Babcock test at 60° when corrections are made for the true density of the fatty materials

1939	Jersey				Holstein			
	Fat	Density (60°)	Corrected	Error	Fat	Density (60°)	Corrected	Error
	%		%	%	%		%	%
Jan.	5.6667	0.8943	5.6308	+0.0359*	3.0208	0.8970	3.0107	+0.0101
Feb.	4.9312	0.8968	4.9137	+0.0175	3.0469	0.8971	3.0371	+0.0098
Mar.	5.4650	0.9004	5.4674	−0.0024†	3.0250	0.8986	3.0203	+0.0047
Apr.	5.1125	0.8945	5.0812	+0.0313	3.2938	0.8982	3.2872	+0.0066
May	5.1375	0.8965	5.1175	+0.0200	3.3417	0.8964	3.3283	+0.0134
June	5.0375	0.8981	5.0269	+0.0106	3.1458	0.8996	3.1444	+0.0014
July	5.0375	0.8962	5.0162	+0.0213	3.1188	0.8950	3.1014	+0.0174
Aug.	5.3833	0.8936	5.3450	+0.0383	3.2656	0.8960	3.2511	+0.0145
Sept.	5.6958	0.8931	5.6521	+0.0437	3.5792	0.8928	3.5506	+0.0286
Oct.	5.1844	0.8902	5.1279	+0.0563	3.7750	0.8898	3.7322	+0.0428
Nov.	5.1938	0.8919	5.1471	+0.0467	3.1406	0.8914	3.1106	+0.0300
Dec.	4.9625	0.8917	4.9168	+0.0457	3.0042	0.8924	2.9788	+0.0254

* Overreadings, positive.
† Underreadings, negative.

DISCUSSION

These data indicate that the density of the purified milk fat is uniformly constant and is not affected, significantly, by breed, season or feed. The density of the materials from the Babcock test estimated as fat showed greater variations that may have been due to the method of obtaining the fatty materials, to inherent variations that occurred in conducting the Babcock test or to inevitable changes in the chemical composition of the milk fat. The density figures for the fatty materials are minimum rather than maximum values, because in aspirating these materials from the neck of the test bottles it was not possible to obtain the film of water next to the glass.

The amount of this watery material is greater at the edges near the lower part of the fat column than at the middle or near the top. The fatty materials estimated as fat have a density of less than 0.9 at 60°. If these materials reach an equilibrium temperature of 60° within five minutes, then a fundamental error is introduced in the Babcock test for milk and for cream, because the estimations would be too high. It may be assumed that this deviation in density below 0.9 is within the limits of error in reading the fat column. However, if the test bottles are more finely calibrated, and if more precise methods are used in reading the Babcock test, then these differences in density would be of some significance.

The coefficient of expansion of the purified milk fat and of the materials estimated as fat is practically constant within the temperatures studied, with the purified fat having the highest value.

SUMMARY AND CONCLUSIONS

1. The density of the purified milk fat in this study was relatively constant and was not affected to any marked extent by breed, season or feed.
2. The density of the fatty materials estimated from Babcock test as fat is higher than that of the corresponding purified fat, but for both types of fat is less than 0.9 at 60°; this introduces a fundamental error in the Babcock test.
3. The coefficient of expansion of the purified fat averaged 78.34×10^{-5} and of the material estimated as fat, 75.58×10^{-5}.

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THE PHOSPHATASE TEST AS USED BY THE MASSACHUSETTS DEPARTMENT OF PUBLIC HEALTH FOR LAW ENFORCEMENT PURPOSES*

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The April, 1942, number of the JOURNAL OF DAIRY SCIENCE contains a statistical article by L. H. Burgwald (2) upon "The Phosphatase Test—Extent of Use in North America," from an address presented at a meeting of the American Public Health Association, October 16, 1941. The following is quoted from the article:

"One state laboratory stated that they used the Scharer Field and Laboratory tests for detecting underpasteurization, and used the findings as a basis for prosecution.

"The answers to question 10, 'Have you noticed any decrease in the number of samples improperly pasteurized as compared to the first time tests were made?' indicates that the phosphatase test has been of decided value in insuring proper pasteurization. One hundred thirty-seven answered 'Yes' while 32 answered 'No.' The majority of those stating the amount of decrease reported that it is more than 50 per cent."

In the spring of 1939 I was informed, much to my surprise, that apparently only in the State of Massachusetts was the phosphatase test used for the purpose of prosecuting persons who represented raw milk or incompletely pasteurized milk as pasteurized. The results of the questionnaire by Burgwald indicate that apparently there has been no change in this situation during slightly more than two years, for Massachusetts is the State referred to in the above article. During the discussion of Burgwald's paper a statement was made to the effect that a positive phosphatase reaction should not be used as a basis for prosecution. My associate, E. B. Boyce, then outlined a certain specific instance of a violation discovered by the use of this test which resulted in the prosecution and conviction of the responsible person. Subsequently during the discussion a representative from Florida stated that he also had used the phosphatase test for prosecution purposes (1). It seems improbable that only in Massachusetts is this test used to prosecute persons for fraudently selling a potentially dangerous article bearing a safety label. There has recently been published a statement that the Division of Health of Dayton has secured a conviction through the use of the phosphatase test (3). In Massachusetts the test is used for law enforcement purposes not only by the State Health Department, but also by the milk inspectors of some of the cities.

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In 1937 the Massachusetts Department of Public Health secured 16 convictions for representing as pasteurized certain milk not heated to a temperature of at least 142° F. and held at such temperature for not less than 30 minutes. The evidence in 8 of these cases was direct evidence, but the evidence in the balance was secured by the phosphatase test. In 1938 a systematic drive was made against those who were suspected of "cheating" which resulted in 42 convictions. In 1939 there were 19 convictions; in 1940 there were 14 convictions; and in 1941 there were but 9 convictions. The validity of the phosphatase test was not questioned in any of these cases. In 1938 the phosphatase test was applied to 3,117 samples of bottled milk and cream represented as pasteurized, of which 112 or 3.59 per cent gave positive reactions. In 1941 the test was applied to 3,679 similar samples of which 35 or 0.95 per cent were positive. This represents a reduction of nearly 71 per cent in the number of improperly pasteurized samples in a four-year period. The reactions classed as positive for the purpose of the above computations include all reactions ranging from those produced by straight raw milk up to and including doubtful reactions. A recent report from a state, showing no prosecutions for this type of offence during 1941, states that of the samples represented as pasteurized "19 per cent were found to be either raw or underpasteurized when examined by the phosphatase test."

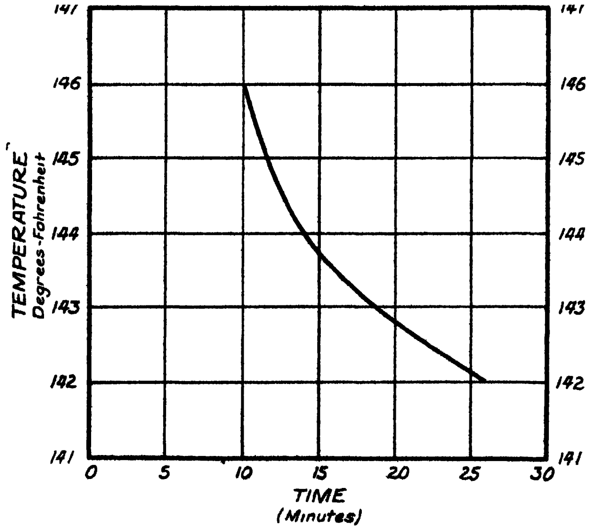
In view of the apparent situation regarding prosecutions, the Massachusetts procedure may be of interest. Prior to the adoption of the phosphatase test evidence of violations of this character could be obtained only with difficulty. In some instances, convictions were obtained by evidence showing that the recording thermometer chart had been rotated, thereby giving a false impression that the milk had been held at the pasteurization temperature for the proper time. In other instances, the mercury thermometer was inaccurate, the scale apparently having been adjusted at the pasteurizing plant. If the inspectors happened to arrive at an establishment under suspicion as the milk was being heated, they would stay at the plant long enough to time the recording thermometer. They would then leave the plant before the milk reached the pasteurizing temperature and would return in about twenty minutes. Occasionally, during this period, the milk had reached the pasteurizing temperature, had apparently by the thermometer chart been held at that temperature for thirty minutes, and much of it had been bottled. By means of this and similar procedures, thirteen convictions were secured in 1935, one in 1936, and eight in 1937.

Prior to its adoption for official work, the phosphatase test was studied for several months, using laboratory pasteurized milk, milk collected for bacterial examination on efficiency tests, commercial milk obtained from dealers known to be reputable, and also on milk collected from dealers suspected to be otherwise. The first case was tried on September 23, 1937, in the District Court of Eastern Essex, sitting at Gloucester, Massachusetts. The defendant entered a plea of "not guilty" but admitted a finding of "guilty." The judge then stated that he would hear the case in his lobby after adjournment. The judge gave the chemist a thorough cross-examination upon the merits of this new test. The defendant's lawyer also had an opportunity to do likewise. The judge then imposed a substantial penalty which the defendant paid. This probably was the first presentation in any court of the United States of this method of analysis.

In no case prosecuted have we been able to find faulty apparatus, etc., the cause for improper pasteurization. But in our opinion, the condition or conditions found were due to deliberate violation by someone in authority at the plant who had no misgivings in selling unpasteurized milk or raw milk as pasteurized. The following conditions responsible for the positive reactions by the phosphatase test have been found: putting pasteurized labels on containers of raw milk; bottling pasteurized milk subsequently to raw milk without washing the cooler, pipe lines and filler; adding raw milk to a vat of pasteurized or nearly pasteurized milk during the holding period or while drawing off; operating without a leak escape valve or plugging the leak escape outlet in a leak escape valve with the effluent piping connected; catching the drip from the leak escape valve and putting it into the vat before drawing off; drawing off prior to the expiration of the holding period but keeping the temperature up so that the chart would show legal pasteurization; rotating the recording thermometer chart; pasteurizing at a low temperature and making, on the recording thermometer chart, a false record of the corresponding readings of the mercury and recording thermometers during the holding periods; lowering the metallic scale of the mercury thermometer; buying pasteurized cream containing 40 per cent fat and reducing it to 36 per cent by the addition of raw skim milk or raw whole milk; and by separating unsold and returned bottled milk, both raw and pasteurized, without exercising sufficient care to exclude all the raw milk from the mixture to be separated. Proprietors of plants where such conditions existed have been prosecuted.

The routine procedure first employed by the Department was developed for the purpose of answering all probable as well as improbable questions and criticisms that might be raised against the test by any attorney or his expert witness. Experience has shown that it is not always necessary to follow this entire procedure, but it is usually advisable to make the efficiency test. The procedure is as follows: The inspectors collect samples in the usual manner from milk wagons, stores, and pasteurization establishments. When the milk reaches the laboratory, the Scharer (4) modification of the phosphatase test, 10 minutes' incubation, is applied to all samples represented as being pasteurized. The Scharer modification of the phosphatase test is preferred first because it saves considerable time and consequently hastens the supplemental and follow-up work, and second because it is more easily explained to and understood by the judge and the jury, if any, than would be the phenol number. If the phosphatase test shows incomplete pasteurization, it is repeated, usually for a longer incubation period, upon another portion of the milk, and if positive, there is added to a third portion of the milk the reagent used for detecting the presence of phenol. If phenol is shown to be absent, it is therefore, very evident that there is no phenolic body in the milk which will give the reaction. Another portion of the milk is placed in a test tube and heated to 142° F. One-ml. portions are removed every five or ten minutes during a thirty-minute period and are immediately placed in the cold substrate solution. Each of these portions is then incubated and after incubation is tested for the presence of phenol. If there is nothing in the milk which will liberate phenol by heating to 142° F. for thirty minutes, at least one of the above samples will show a negative phosphatase test. This will also give an indication of the extent of under-pasteurization, if any.

TIME OF
INACTIVATION OF THE PHOSPHATASE IN MILK
DURING COMMERCIAL PASTEURIZATION



NOTE — Samples taken at 5minute intervals

As soon as possible after a violation has been found, the inspectors go to the plant where the suspected milk was pasteurized and perform an efficiency test during the usual operation of pasteurization. Samples of the raw milk are obtained, a sample is obtained when the milk reaches 142° F., samples are obtained during the holding period at intervals of five or ten minutes, a sample is taken from the outlet of the cooler, and from the bowl of the filler.

The first bottle from the filler is taken, and another bottle is taken ten or fifteen minutes later. If possible, there is obtained a bottle of milk pasteurized the prior day, which sample frequently gives a positive test. These samples are all tested by the phosphatase test, and in most instances, bacteriological examinations are also made.

In all but two of the efficiency tests, the phosphatase had been inactivated in less than thirty minutes. In each of these two cases, the metallic scale of the mercury thermometer had been so changed that the thermometer read three degrees high and consequently the milk was not pasteurized as defined by law.

After the efficiency test is made, the person involved is given an opportunity to be heard, and then he is asked the impossible, namely, to explain why the milk was properly pasteurized when performed in the presence of an inspector of the Department, and why it was improperly pasteurized at least once when an inspector of the Department did not observe the process.

It is possible that the length of time required to heat the milk up to the pasteurizing temperature has some bearing upon the time at which the phosphatase is inactivated during the holding period. If the milk is heated very quickly, as is done in laboratory experiments, the time of inactivation may necessarily be longer than if the milk is heated more slowly as is the case in some commercial pasteurizing establishments.

The chart compiled from many efficiency tests made in commercial plants, where as far as possible complete details as to temperature and time have been obtained, shows the average time at which the phosphatase was inactivated at temperatures of 142°, 143°, and 144° F., but for temperatures from 145° to 148° F. it shows the average temperature at which the phosphatase was inactivated within ten minutes. In construing this chart, it should be understood that the samples were taken at five-minute intervals, and therefore, in all instances, the phosphatase had become inactivated in somewhat less time than is indicated on the chart.

CONCLUSION

Violations of the Massachusetts law by representing as pasteurized raw or incompletely pasteurized milk have been materially reduced by means of the phosphatase test, supplemented, when obtainable, by other evidence and followed by prosecutions.

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THE OSCILLATORY CHARACTER OF THE VARIATIONS IN THE WEIGHTS OF DAIRY HEIFERS

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Studies in animal growth are often complicated by wide and rapid daily variations in body weight. Although the investigator is primarily interested in the organized body tissues there are, in addition, water and food recently consumed, partially digested food and waste materials which are responsible for most of the large and rapid changes in total weight of the body. These may mask to a considerable degree the true changes in the organized body tissues. An understanding of the nature of these variations should be of considerable value in properly evaluating results of investigations in the growth of dairy heifers.

Lush *et al.*, (3) discussing the accuracy of beef cattle weights assumed that consecutive daily weights on the same animal were independent in a probability sense. Baker and Guilbert (1), however, showed that a significant correlation exists between the deviations of consecutive daily weights from trend for yearling beef cattle. Maymone and Sircana (4) examined the weights of mature dairy cows that were lactating and found that the daily weights consisted of a linear trend for periods up to 75 days plus an oscillatory part suggestive of a sine curve.

The purpose of this paper is to present data for a particular non-roughage experiment which show that the weights of growing dairy heifers consist of a general trend plus an oscillatory variation. The character of the oscillatory part of the variation in the daily weights changes with the age of the heifers. For young heifers the daily weights are pronouncedly below or above the trend for long periods of time. When the heifers are older the weights are not so pronouncedly above or below trend and may change from above trend to below trend within short periods. The variability of the weights of these heifers is about constant from 3 to 10 months of age and then suddenly increases. Because the periods of oscillation decrease for older heifers and the variability of daily weights increases, the oscillatory nature of the deviations from trend is increasingly obscure but still definite. The daily weights as the heifers become older behave in a manner very similar to those for yearling beef animals already reported (1). The frequency distributions of deviations from trend are compared with normal.

DATA

The data consist of three consecutive daily weights taken at ten-day intervals from 100 to 437 days of age on Holstein heifers that were born on

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TABLE 1

Weights, mean weights and pooled variances for 9 Holstein heifers born 6–3–1930 for 15 three-day periods at ten-day intervals

Date 1930		Calf number									Mean	Pooled variance
		33E	34E	35E	36E	37E	38E	39E	40E	41E		
Sept.	13	190	170	166	152	114	186	184	166	158	168.07	13.61
	14	200	172	166	152	116	190	190	170	156		
	15	200	178	168	156	124	192	192	170	160		
Sept.	23	204	184	180	174	120	192	192	184	182	180.74	6.33
	24	210	184	182	174	122	190	192	186	184		
	25	210	192	184	178	124	192	194	188	182		
Oct.	3										197.22	15.78
	4	226	210	206	202	126	210	208	198	200		
	5	222	210	208	202	112	204	212	198	196		
Oct.	13	222	224	210	210	126	210	214	202	204	204.44	9.17
	14	228	224	212	212	130	222	214	202	200		
	15	226	228	214	214	126	218	216	206	206		
Oct.	23	264	240	228	230	146	230	220	212	220	220.30	9.11
	24	256	236	224	230	144	230	214	212	220		
	25	260	238	232	228	144	230	226	216	218		
Nov.	2	270	244	240	228	152	230	228	214	224	227.56	15.50
	3	260	250	240	234	150	242	230	214	230		
	4	266	252	238	234	152	242	230	220	230		
Nov.	12										244.89	4.00
	13	292	262	250	248	160	258	244	240	242		
	14	298	260	252	250	160	262	246	240	244		
Nov.	22	304	268	264	262	164	278	266	262	262	260.89	41.78
	23	316	286	262	266	174	266	270	262	264		
	24											
Dec.	2	332	292	280	290	180	272	280	284	284	275.00	12.00
	3	324	295	279	292	178	274	279	280	274		
	4	320	290	280	286	176	274	277	275	278		
Dec.	12	348	304	294	300	182	284	290	282	288	286.33	11.33
	13	340	301	298	301	184	282	298	288	290		
	14											
Dec.	22	340	316	310	308	192	302	302	296	306	299.41	12.28
	23	350	316	312	306	194	310	304	296	304		
	24	350	320	316	310	198	306	310	300	310		
1931												
Jan.	1	370	328	330	318	198	302	322	302	316	313.26	23.39
	2	378	336	328	316	200	312	324	308	320		
	3	378	338	324	322	206	320	328	310	324		
Jan.	11	378	340	352	306	212	324	340	320	334	323.55	30.50
	12	376	344	348	326	216	324	350	322	336		
	13	374	340	342	320	216	312	344	310	330		
Jan.	21	370	354	362	318	220	304	352	310	340	329.30	25.56
	22	380	360	361	320	224	306	362	313	343		
	23	388	366	361	314	228	308	362	323	342		
Jan.	31	360	350	372	316	244	290	356	314	352	327.44	18.00
Feb.	1	368	344	370	316	238	298	354	304	348		
	2											

TABLE 1—(*Continued*)

Date 1931	Calf number									Mean	Pooled variance
	33E	34E	35E	36E	37E	38E	39E	40E	41E		
Feb. 10	398	346	388	302	246	294	378	320	368		
11	402	358	390	310	252	290	382	322	368	340.30	11.83
12	400	354	388	304	256	296	382	324	370		
Feb. 20	400	364	406	312	276	308	406	336	370		
21	406	380	412	310	276	308	406	336	372	354.81	13.83
22	404	368	412	310	274	316	408	332	372		
Mar. 2	436	392	414	332	290	324	420	348	380		
3	438	398	420	334	288	318	424	344	382	371.52	21.11
4	432	391	426	340	281	332	429	340	378		
Mar. 12	462	418	442	352	290	324	454	354	376		
13	466	410	444	346	284	332	464	356	384	386.56	21.55
14											
Mar. 22	474	434	460		298	334	470	354	394		
23	474	424	452		292	326	464	356	382	297.50	28.81
24	472	422	454		294	322	464	350	374		

the same day. There were 9 heifers for the first 193 days and 7 of the same heifers for an additional 143 days. The daily weights, means of all weights for each three-day period, and a pooled estimate of the variance for each three-day period are given in tables 1 and 2.

These heifers received a non-roughage diet consisting of a mixture of ground barley, soy bean meal, wheat bran, calcium carbonate and sodium chloride supplemented with cod-liver oil. From three to ten months of age their average body weights were approximately 80 per cent of Eckles' (2) normal. After reaching ten months of age, they rapidly approached and finally exceeded normal weight at fifteen months of age.

Since these animals bloated frequently, their weight variations may be somewhat different than for heifers fed a normal diet containing roughage. However, as has been pointed out, at the later ages the weights of these heifers behaved very similarly to the weights of yearling beef cattle fed on a normal alfalfa hay ration (1).

TREATMENT OF DATA

A preliminary examination of the means and variances presented in tables 1 and 2 indicated that some fairly abrupt change in the character of the daily weights took place about April 1, 1931. At that time the rate of daily gain increased and the variability of the daily weights markedly increased. For these reasons the data were broken into the two periods. The data were extended as far as possible until there was again a change in the character of the daily weights.

Straight lines were fitted by means of the least-squares technique to the weights of each heifer for each of the two periods. Several people with extensive experience in curve fitting judged the straight lines to adequately

TABLE 2

Weights, mean weights and pooled variances for 7 Holstein heifers born 6–3–1930 for 15 three-day periods at ten-day intervals

Date *1931*		Calf number							Mean	Pooled variance
		33E	34E	35E	37E	38E	39E	40E		
April	1	500	452	482	330	384	484	370	423.81	157.71
	2	498	450	492	314	330	488	366		
	3	504	454	492	316	332	496	366		
April	11	530	472	498	334	350	518	392	442.10	30.36
	12	540	462	496	328	344	516	386		
	13	542	482	506	336	350	514	388		
April	21	546	494	558	348	370	540	412	460.00	207.50
	22	532	472	510	352	358	536	402		
	23	536	490	540	350	358	544	412		
May	1	530	496	542	372	382	552	398	470.10	60.78
	2	550	488	550	368	384	548	420		
	3	546	492	530	370	392	544	418		
May	11	536	502	554	374	390	562	434	482.19	22.78
	12	538	496	562	382	396	580	440		
	13	550	502	554	380	390	566	438		
May	21	580	504	564	402	428	594	464	507.43	18.86
	22	586	514	568	406	424	598	472		
	23									
May	31									
June	1	560	524	600	428	430	612	486	522.43	54.00
	2	562	542	606	428	448	604	484		
June	10	564	556	604	450	460	624	492	534.43	53.43
	11	570	532	612	452	460	616	491		
June	20	566	550	634	450	444	612	482	539.62	116.50
	21	552	558	630	456	468	618	490		
	22	582	562	616	464	480	626	492		
June	30	576	574	612	472	468	624	508	544.76	57.64
July	1	588	542	606	470	454	628	510		
	2	574	556	614	474	456	628	506		
July	10	626	570	642	492	480	660	550	568.14	119.71
	12	596	562	626	496	468	650	536		
	13									
July	20	634	562	638	500	520	658	568	591.24	232.50
	21	640	592	690	494	534	664	558		
	22	674	592	666	484	532	652	564		
July	30	640	602	646	498	542	676	570	597.48	207.57
	31	660	570	680	506	528	652	548		
Aug.	1	644	600	690	520	540	664	570		
Aug.	9	660	580	700	530	522	654	608	614.29	180.00
	10	670	592	740	526	538	674	606		
	11									
Aug.	19	724	638	722	556	564	674	590	635.14	207.14
	20	724	612	720	550	582	664	606		
	21	680	630	732	538	576	642	614		

account for the general trend in the daily weights within each of the periods. Deviations from trend were computed as the observed weight minus the weight given by the fitted straight line.

The equations of the trend lines for the first period are:

$$
\begin{aligned}
y - 333.6 &= 1.404\ (x - 97.1)\\
y - 303.8 &= 1.255\ (x - 97.1)\\
y - 308.4 &= 1.505\ (x - 97.1)\\
y - 277.3 &= 0.969\ (x - 97.1)\\
y - 197.6 &= 0.999\ (x - 97.1)\\
y - 274.1 &= 0.750\ (x - 97.1)\\
y - 308.5 &= 1.486\ (x - 97.1)\\
y - 275.4 &= 1.001\ (x - 97.1)\\
y - 290.2 &= 1.247\ (x - 97.1)
\end{aligned}
$$

and for the second period are:

$$
\begin{aligned}
y - 585.2 &= 1.232\ (x - 70.4)\\
y - 535.5 &= 1.133\ (x - 70.4)\\
y - 600.6 &= 1.604\ (x - 70.4)\\
y - 432.4 &= 1.634\ (x - 70.4)\\
y - 446.4 &= 1.617\ (x - 70.4)\\
y - 598.9 &= 1.255\ (x - 70.4)\\
y - 485.2 &= 1.685\ (x - 70.4)
\end{aligned}
$$

where y is weight in pounds and x is the number of days from the beginning of the period.

RESULTS

The deviations from trend show a pronounced tendency to be all positive or negative for each three-day period for each of the two longer periods. The general situation is illustrated in figures 1 and 2.

If sets of three are drawn at random from a population with equal numbers of positive and negative deviations, then it is expected that $\frac{1}{4}$ of all such sets of three are all alike in sign and $\frac{3}{4}$ of all such sets have two like signs. For the data of table 1 counting only complete sets we find 105 with signs all alike and 20 with 2 signs alike. The probability of this result occurring by chance is vanishingly small.

For the data of table 2 we find 33 sets of 3 with signs all alike and 47 with 2 signs alike. The probability of this occurring by chance is less than 1 in 100.

Not only do the data tend to fall above or below the trend line in sets of three but adjacent sets of three tend to fall in the same direction from the trend lines producing very pronounced oscillatory motions.

The extent of variations in the deviations of daily weights due to changes which affect all animals alike and to changes that are individual with each animal was studied by analysis of variance. The results are presented in table 3. The day-to-day changes in environment are from 1.5 to 3 times as important per degree of freedom as individual animal differences in producing daily variations in the deviations from trend. It follows from the F-values of table 3 that a significant positive intraclass correlation exists. This correlation coefficient is *.18* for the first period and *.08* for the second period. Daily weight data for yearling beef cattle showed significant intraclass correlations of .10 and .15 for two, thirty-day periods (1).

A comparison of the grouped frequency distributions of the deviations from trend with a normal distribution was made. The standard deviation of the deviations for the first period was 12.65 and for the second period was 15.26. These values are about twice the standard deviations found in (1) for yearling beef animals (8.64 and 6.97 for full and shrunk weights respectively). It was seen that the combined distribution for the two periods did not differ significantly from normal ($P > .05$). The number of negative

TABLE 3

Analysis of variance showing the amount of variation in daily deviations of weight due to common and individual causes

Source of Variation	Period I Degrees of Freedom	Sum of Sq.	Mean Sq.
Regressions	18		
Days	52	20,098.69	386.51
Groups (within)	413	54,391.40	131.70
	F = 2.93		
	P < .01		

Source of Variation	Period II Degrees of Freedom	Sum of Sq.	Mean Sq.
Regressions	14		
Days	38	13,116.45	345.17
Groups (within)	228	49,092.39	215.32
	F = 1.60		
	P between .05 and .01		

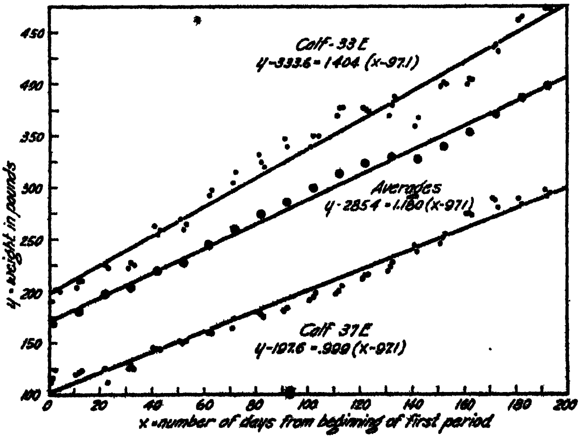

FIG. 1. Linear trends fitted to the daily weights of two heifers and to the averages of nine heifers for ages from 100 days to 300 days.

deviations (393) is not significantly different from the number of positive deviations (370). However, the variance for the first period (160.21) is significantly less than for the second period (233.09), ($P < .01$) but not nearly as much less than as would be expected from the pooled variances given in tables 1 and 2. More of the variance is due to oscillatory variation in the first period than in the second. The distribution of the deviations from trend are nearly normal as is generally expected in biological work. However, these deviations must be considered as being made up of two distinct parts, one due to a somewhat regular oscillatory movement of varying length and the other due to accidental or irregular causes.

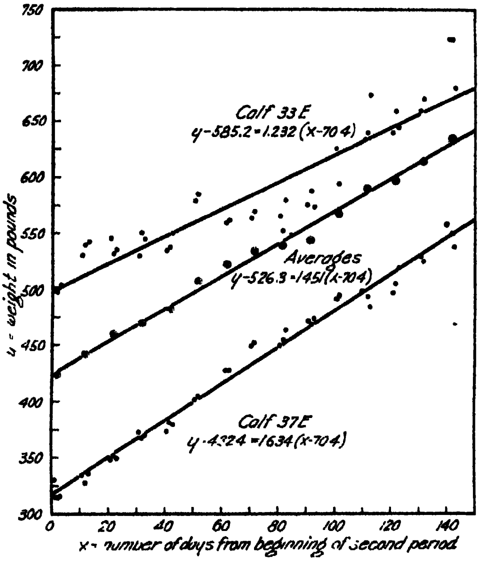

FIG. 2. Linear trends fitted to the daily weights of two heifers and the averages for seven heifers for ages from 300 days to 450 days.

SUMMARY

Data are presented showing that the daily weights of growing dairy heifers receiving a non-roughage diet consist of a general trend plus an oscillatory function of time which tends to become less pronounced as the heifers increase in age and size. The behavior of the weights of these heifers when older was very similar to the behavior of the weights of yearling beef heifers fed on a normal alfalfa hay diet which indicates that the different behaviors at different ages observed for these heifers was a matter of age and not of diet. At any age there was always a tendency for a deviation from trend in a daily weight to depend on the deviations for the adjacent days. This fact must be considered in calculating the accuracy of the results of feeding trials. If weights that are used as the basis of conclusions in feeding trials happen to fall at the right parts of the natural oscillations, then very misleading results may be obtained.

From the data presented it appears that for short time trials several animals should be included if possible, the environmental conditions be kept uniform, and frequent weighings throughout the trial should be made. For long-time trials, regular comparable weighings as often as possible should be made throughout the entire course of the trial.

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THE HERITABILITY OF BUTTERFAT PRODUCTION IN DAIRY CATTLE[1]

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The present study grew out of an earlier inquiry[2] into the effects which selection of dams might have on sire indexes. That study showed the importance of knowing the degree to which the observed variations in a character are hereditary, if one is to predict the consequences of various breeding plans or to discount the effects of selection or other practices on sire indexes and on estimates of a cow's breeding worth. It also indicated that the heritability of differences in annual butter fat production was similar in two different kinds of data (Iowa D.H.I.A. prior to 1937 and Holstein-Friesian H.I.R. prior to October, 1938) and was not very different from that indicated by several other studies. This suggested that heritability of butterfat production might be nearly enough constant in all cattle populations which are of much interest to dairy breeders, that there would be little error in using a single figure for it when estimating the probable results of alternative breeding procedures.

The present investigation was focussed mainly on the question: In the data currently being used for proving dairy bulls, how heritable are the differences between the butterfat records of cows which are mated to the same bull? Questions of whether this really differed from breed to breed, and of the size and heritability of the differences found between herd averages arose as incidental to the main object.

By "heritability" is meant the extent to which observed differences between individuals are caused by differences in the heredity which they have. But because differences in heredity cannot be measured directly, heritability must be estimated indirectly from the differences observed between individuals related in such a way that their probable genetic differences can be computed from the laws of inheritance. The best relationship for this purpose generally is that between parent and offspring. Doubling the intra-sire regression of daughter's record on dam's record seems the most dependable method for estimating heritability in data like these, where the sire cannot express the characteristic himself, where the dams are likely to have been a bit more highly selected than the daughters, and especially because feeding and other management practices are almost certain to have differed considerably from herd to herd.

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[1] Journal Paper No. J-1018 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 317.

[2] Lush, Jay L., Norton, H. W. III, and Arnold, Floyd. 1941. Effects which selection of dams may have on sire indexes. JOUR. DAIRY SCI., 24: 695–721.

Computing the daughter-dam regression on an intra-sire basis restricts the analysis to the heritability of those differences which existed among the mates of a sire. The extent to which differences between one group of mates and another such group (usually in a different herd) are caused by differences in their average heredity cannot be measured directly by this method. Since most sires are proved in only one herd, the intra-sire basis automatically excludes from the likeness between daughter and dam most of the environmental contributions. Those sires which were proved in two or more herds presumably tend to have been used in herds with somewhat similar standards of feeding and management, as in partnerships.

Since a daughter gets only a sample half of the inheritance her dam has, the regression of daughter on dam must be doubled to estimate what fraction of the differences between the records of the mates of a sire were due to differences in the heredity of those mates. This answers well enough for the average effects which each gene had in the various combinations of genes with which it was associated in that group of mates and daughters. These average effects are what is meant when we call a gene "good" or "bad," or speak of it as "a gene for" high or low fat production. But if two or more genes have when together an effect which is greater or less than the sum of their average effects in that population, these differences between the actual and the expected effects[3] are transmitted to the offspring only as far as the whole group of genes necessary for each such joint effect is transmitted intact. Thus dominance deviations are not transmitted from parent to offspring, since only one gene of the pair concerned can be transmitted in any one gamete. Interactions requiring the simultaneous presence of two non-allelic genes would be transmitted to only one fourth of the offspring (in a population mating at random), interactions requiring three such genes would be transmitted to only one eighth of the offspring, etc. Thus the method of doubling the intra-sire regression of daughter on dam gives an estimate of heritability which includes all of the additive effects of genes, none of the dominance deviations, and something less than half of the effects of the non-linear interactions of non-allelic genes. If the non-linear interactions and dominance deviations of genes are abundant and large, the estimate of heritability derived from the daughter-dam regression will be somewhat lower than an estimate based on likeness of identical twins, even after the latter is freed from the effect of common environment.

SOURCE AND DESCRIPTION OF DATA

The data consisted of the 2154 daughter-dam comparisons used in proving 283 sires in Iowa Dairy Herd Improvement Associations during the period Jan. 1, 1936 to Dec. 31, 1939.[4] Only the pounds of fat produced in the first 305 days of the lactation were studied. All records were corrected for age and were on the basis of twice-a-day milking. The average number of daughter-dam comparisons per sire was 7.6, the minimum of course being five. Only a few sires were proved by more than 10 daughter-dam comparisons and very few indeed by more than 20. The fat production figures for both dam and daughter were averages of all normal lactation records available.

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[3] Such differences are called epistatic or interaction effects if they concern combinations of non-allelic genes, or dominance deviations if they concern two allelic genes.

[4] The investigation was begun early in 1940.

RESULTS

The average productions, the pooled intra-sire correlations and regressions of daughter on dam, the number of daughter-dam comparisons, and the number of sires in each breed are listed in table 1.

TABLE 1

Averages and intra-sire correlations (r) and regressions (b) of daughters on dams, separately by breeds

Breed	Ave. dams' production	Ave. daughters' production	r	b	No. of pairs	No. of sires
Holstein	384.7	388.9	.130	.133	1003	116
Guernsey	366.9	364.8	.147	.147	390	53
Jersey	373.4	389.7	.166	.157	351	52
Brown Swiss	372.1	362.7	.076	.085	269	40
Ayrshire	349.4	360.0	.270	.208	70	11
Shorthorn	288.0	272.1	.046	.045	58	10
Red Polled	346.2	213.2	.084	.051	13	1
Average (intra breed)			.134	.134	2154	283

The intra-sire daughter-dam regressions varied somewhat from breed to breed but these differences were not statistically significant. The figures hint that the management in the dual-purpose herds may be such that the cows do not show their innate capacity as clearly as in the more highly specialized breeds. The data, however are so few that this is only a hint. Perhaps it would not be confirmed by more extensive evidence.

Since the differences between breeds were not significant the data were pooled, considering them as a homogeneous intra-sire population. The pooled regression and correlation were both .134, based on 1870 degrees of freedom.

These figures describe the present data, in which the average number of lactations in the dam's record was 3.15 and the average number of lactations in the daughter's record was 1.68 In averages of two or more lactations per cow the differences due to circumstances which change from lactation to lactation will tend to cancel each other, thus decreasing the environmental variance but leaving the genetic variance unchanged. For comparing these findings with others and making them useful for generalizing to cases where each cow has n records, it is desirable to express these findings in terms of what they would be if each cow had only one record. Let *b* equal the regression of daughter on dam when single lactation records of each are used, and *b′* equal the regression when lifetime averages are used. Then if all dams had the same number (m) of the lactation records: $b = b' \left[\frac{1 + (m-1)r_{dd}}{m}\right]$ where r_{dd} is the repeatability; i.e. the average correlation between successive lactations of the same dam. But where *m* is variable, as it was in the present data, *b* is related to *b′* approximately thus[5]:

$$b = b' \left[\frac{1 + (m-1)r_{dd}}{\bar{m}} + \frac{\sigma^2_m(1 - r_{dd})}{\bar{m}^3}\right]$$

On the basis of other studies, the repeatability within herds (r_{dd}) was assumed to be .4. The variance of *m* in these data was 3.09. Consequently *b* had a value of .087. Doubling this yields .174 for heritability.

The pooled intra-sire regression coefficients for sires having daughters in only one herd was .108 and for sires having daughters in two or more herds was .155. Both regressions were significant and the difference is in the direction expected, but it is not statistically significant on this amount of data. If the .108 is taken at face value and the intra-herd repeatability of single records is + .4, the heritability of differences in single records is .140 instead of .174 as was computed from all intra-sire records. Apparently the latter contained some environmental correlations between daughter and dam in those cases where a sire was proved by records from more than one herd.

The correlation and regression coefficients in table 1 are nearly equal, whereas it had been expected that the correlation would be distinctly smaller. The relation between the two statistics is:

$$b = r\,\frac{\sigma_o}{\sigma_d}.$$

It was expected that σ_d would be smaller than σ_o since it was presumed that the dams were more highly selected than the daughters. Moreover the dams averaged 3.15 lactations each and the daughters only 1.68 lactations. This should have made the averages of the dams less variable. However, the daughters were more nearly contemporaneous than the dams and therefore, within the group pertaining to each sire, probably did not encounter such a wide range of year-to-year changes in environmental conditions as the dams did. Within each group the daughters were more closely related to each other than the dams were. This would have tended to make the intra-sire standard deviation of the daughters a little less than that of the dams, as will be discussed later. Differences in age should not have caused differences in standard deviations, since the age corrections, being multiplicative, would cancel the tendency for standard deviations in uncorrected records to increase with age of cow. Why the intra-sire standard deviations of daugh-

[5] We are indebted to Professor W. G. Cochran for this correction for variation in *m*.

ter and of dam are so nearly equal is not clear. It may have been merely a coincidence that these opposing tendencies so nearly cancelled each other.

INTRA-BREED REGRESSIONS

When each of the different breeds was studied as a homogeneous population, rather than on an intra-sire basis as was done in table 1, the correlations and regressions were as shown in table 2. The figures are 2 to 3 times as large as the corresponding coefficients on an intra-sire basis. The differences between breed regressions are significant. This must be due to greater herd-to-herd heterogeneity in the management of some breeds as compared to that of others. Greater genetic heterogeneity within some of the breeds is not wholly excluded, but studies of the breeding systems used in different breeds have shown, in all of them yet studied, only a little separation into non-interbreeding families or strains. Heterogeneity of ideals with respect to the level of production desired may have been slightly more extreme in one breed than in another.

TABLE 2

Daughter-dam correlations and the regression of daughter on dam, each breed being treated as a homogeneous unit

Breeds	r	b
Holstein	.324	.329
Guernsey	.325	.372
Jersey	.495	.576
Brown Swiss	.229	.254
Ayrshire	.637	.604
Shorthorn	.016	.014
Polled	.084	.051

The magnitude of herd differences is shown in table 3 where the total variance of the population has been analyzed[6] into that portion (B) due to breed differences; that (G) from differences between groups mated to (or sired by, in the case of daughters) the same sire within a breed; and that (C) from differences between cows in the same group.

Group differences (G) are 30 and 35 per cent respectively of the total variance (C + G + B) of dam and daughter productions. Only indirectly can we get even a rough estimate of what portion of G is genetic. Groups of dams mated to one sire are generally related a little to each other because such a group will contain some sets of half and three-quarter sisters and even a few daughter and dam or full sister pairs. For example, the average relarelationship within groups of dams would be around 8 to 12 per cent if one-third of them are half-sisters, another third are three-quarter sisters, not related to those half-sisters, and there are a few daughter-dam or full sister

[6] For method see: Winsor, C. P., and Clarke, G. L., in Jour. Marine Research 3(1): 25–27. 1940.

pairs. The genetic variance among mates of a sire would lack this much of equalling the genetic variance within a truly random sample of dams in the breed. In the present figures the genetic portion of the variance among mates of a sire was .268 (twice the regression) of an actual variance of 3868, which would be 1037. If the average relationship (r) among mates was .10, the genetic variance in a truly random sample of dams from the whole population would be $\frac{1}{1-r}$ of this, or 1152. The variance (G) due to differences between group averages was 1791 and only 115 of this (1152 minus 1037), or about six to seven per cent of it is thus estimated to have been genetic. It is believed that by far the greater part of G arises from differences in environment and management from herd to herd.

TABLE 3

Analysis of variance among records of daughters and dams

Source of variance	d/f	Mean square	Components of variance		
			Kind	Amount	Fraction of total
Daughters:					
Between breeds	6	219864**	B	776	.11
Between groups within breeds	276	22727**	G	2482	.35
Within groups	1871	3838	C	3838	.54
Total	2153	6862	C + G + B	7096	1.00
Dams:					
Between breeds	6	102983**	B	337	.06
Between groups within breeds	276	17498**	G	1791	.30
Within groups	1871	3868	C	3868	.64
Total	2153	5892	C + G + B	5996	1.00

The above estimate is based on the assumption that consanguinity is the only reason for mates of a sire being genetically more like each other than members of a truly random sample of the breed would be. Another conceivable cause for genetic differences between herds is that some assortive mating may be practiced with dairy cattle, irrespective of consanguinity. This would result if high producing animals were brought together in the specialized herds, medium producing in the less specialized ones and low producing in the herds where little attention is paid to the cows, or where the owner does not have financial freedom to buy higher producing stock, or where other qualities than butterfat production are desired. Since no one deliberately tries to breed low producers, it is not believed that this process can have caused *much* genetic differentiation between herds, although there doubtless are some differences in the intensity with which various breeders strive toward the same goal. If there is much assortive mating (or if the average relationship between dams is more than .10), the genetic portion of the variance between group averages is more than estimated above.

DISCUSSION

The heritability value found in this study on an intra-herd basis and for single lactation records is .174. If taken at their face value, the data concerning sires which were used in only one herd indicate that heritability was only .140. This is a little less than has been found in previous studies, which have more usually given values of around .20 to .30. The five per cent fiducial limits for the .174 are .03 and .31. Therefore it is easily possible that the lowness of the value found in the present study as compared with earlier studies is only a sampling variation.

Heritability of differences between cows when each is represented by an average of *n* unselected records would be $\frac{n}{1 - r + nr}$ times as large, where *r* is the intra-sire correlation between records of the same cow. However an increase in *n* decreases the variation between cows. This makes the selection differential smaller, if the percentage of culling remains the same. The net result is that progress from culling on averages of *n* records is $\sqrt{\frac{n}{1 - r + nr}}$ times as large as when culling on only one record each. If $r = .4$, this has values of 1.20, 1.29, 1.35, etc., when *n* is two, three, four, etc.

The inter-group variance was larger among daughters than among dams (35 as compared with 30 per cent of the total variance). About half of this difference can result from the closer relationship of the daughters to each other. If the average relationship among the mates of a sire is .10, the relationship among the daughters becomes .275 if the sire is not related to his mates. This will reduce the genetic variance among the daughters to 835, as compared with 1037 among their dams. The decrease (202) in genetic variance *within* groups of daughters must be balanced by an equal increase in variance *between* groups of daughters. This shift of 202 from variance within groups to variance between groups would be about three per cent of the total of $C + G + B$. Another possible cause for group differences being more important among the daughters is that the dams averaged more lactations each. Differences in feed or management from one year to another thus had more chance to cancel each other in the records of the dams than in the records of the daughters. More often with the daughters than with the dams were all of the records made within a space of two or three years. The dams' records, being spread out over a longer time, offered more chance for seasons when conditions were unusually good to cancel the effects of seasons when conditions were unusually bad. This would tend to make the averages of groups of dams less variable than the averages of groups of daughters.

The breed differences were more important for the daughters than for the dams. The immediate cause for this can be seen in the average productions for each breed as shown in table 1. In the two breeds in which the dams' records averaged the highest, the daughters' averages went yet higher, while in the two breeds in which the dams' records averaged lowest, the daughters' averages went yet lower. Why this happened is not known, but its effects on the variance between breed averages is obvious.

When the two dual purpose breeds are omitted, the breed differences (B) among the means of the five dairy breeds fall to about one sixth of the values shown in table 3 and are therefore comparatively unimportant economically, but they are still statistically significant. These small breed differences remain about twice as large among daughters as among dams.

CONCLUSION

Data on lactation yields of fat in 2154 dam-daughter comparisons used in proving 283 sires in Iowa Dairy Herd Improvement Associations from 1936 to 1939, inclusive, were analyzed for heritability of individual differences and for magnitude of group differences.

Expressed on the basis of records in single lactations, the heritability of differences between cows mated to the same sire was found to be .174 with 5 per cent fiducial limits of .03 and .31 respectively. This .174 is a little less than the .2 to .3 more usually found in other studies.[7] If this .174 is accepted at its face value it indicates that two cows, chosen on the basis of one record each, will probably differ in their breeding values about one-sixth as much as their records differ, and that one selecting cows for high records should expect to find that their breeding values are about one-sixth as far above the average of the group from which they were chosen as their records are.

When only the data pertaining to sires used in but one herd are considered, heritability was .140 instead of .174. The small difference between .140 and .174 was not significant but suggests that environmental correlations contribute a little to the intra-sire likeness between daughter and dam when sires are proved in more than one herd, and that heritability is a little lower than .174.

Heritability was not significantly higher in one breed than in another. There was a faint indication that differences in records corresponded a little less closely to differences in breeding values in the dual purpose breeds than in the more specialized dairy breeds but the difference was far below the level of statistical significance in this volume of data.

Differences between group averages within a breed are mostly due to differences in management or other environmental circumstances, but about 6 to 7 per cent of them are due to differences in the average genetic merit of those groups.

[7] Among very recent studies should be mentioned the findings of A. H. Ward which are summarized on page 37 of the "Seventeenth Annual Report of the New Zealand Dairy Board" for 1940–41. For 3076 daughter-dam pairs, whose lifetime averages were used in proving 104 sires, the intra-sire regression of daughter on dam was about .15 According to whether the number of records per dam was 4, 3, or 2, this is equivalent to a heritability of about .22, .23, or .25 of the differences between single records, assuming that the intra-sire repeatability of records of the same cow was .4.

NORMAL VARIATIONS IN THE AMOUNT OF ASCORBIC ACID IN THE BLOOD OF DAIRY CATTLE*

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The experimental work reported by several investigators (7, 9–11) indicates that dairy cattle are capable of synthesizing sufficient amounts of ascorbic acid in their bodies for normal metabolic functions. Hopkins and Slater (2) are of the opinion that ascorbic acid is synthesized in the small intestines and in the liver. Harris and Ray (1) concluded that the localized concentration of ascorbic acid in the suprarenals was not a reserve storehouse for the body nor a site of synthesis but that it was needed for protecting the normal functional activities of that organ. Whether the high concentration of ascorbic acid in the various tissues is an indication of synthesis at that particular point or whether it is an indication of a requirement for normal functions is not known.

Phillips and associates (5, 6), however, have reported many cases of sterility in bulls and cows that were associated with low concentrations of ascorbic acid in the blood plasma and that a large percentage of "slow breeding" bulls and "hard to settle" cows responded to ascorbic acid therapy. These workers (6) reported that there was a distinct breed difference in the level of ascorbic acid in the blood plasma and that there was also considerable variation in the ascorbic acid level in individual cows. The average ascorbic acid value reported by these investigators was 0.39 mg. per 100 ml. of plasma (range 0.19–0.65 mg.). Wallis (11) obtained blood samples twice monthly from five cows for a period of 10 months and found the average ascorbic acid value to be 0.32 mg. Knight and co-workers (3) reported that the ascorbic acid values of normal cows ranged from 0.43 to 0.62 mg. from the analysis of over 50 samples of blood obtained from four Holstein cows on standard dairy rations.

The relationship between ascorbic acid and the oxidative process in milk has stimulated some investigators to study the effect of the ration on the ascorbic acid content of milk (3, 8). These results indicate that the type of rations fed does not affect the ascorbic acid content of the milk, blood or urine. Knight and associates (3), however, have shown that there is a rapid destruction of ingested ascorbic acid in the rumen. This is interpreted to mean that the feeding of ascorbic acid to cows will not increase the level of this vitamin in the blood.

The apparent relationship between ascorbic acid synthesis, metabolism

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and reproduction emphasizes the importance of obtaining additional information concerning the concentration of this vitamin in the blood plasma of normal dairy cattle. The purpose of this paper is to record the plasma ascorbic acid values obtained from normal calves, heifers and lactating cows and to indicate the variations that are encountered under standard feeding practices.

EXPERIMENTAL

Nineteen calves varying from birth to 12 months of age, 4 heifers and 24 cows of all ages were used in this study. The rations that these animals received corresponded to their respective ages and in all cases they were adequate for maintenance, growth and milk production. The majority of the animals were of the Holstein breed but a few of the other breeds were also included. The breed of all of the animals is included in tables 1 and 2. Some of the cows were open and some were in different stages of pregnancy. Three of the heifers (A29, A31 and C424) and nine cows (A18, A26, D9, 66, 78, 264, 266, 267 and 285) were pregnant during the entire period that samples were taken. Cow A27 became pregnant after the first six samples had been obtained (table 3). All of the rest of the animals were open

A uniform procedure was adopted for the withdrawal of the blood and its disposition after being received in the laboratory. Approximately 20–25 ml. of blood were collected in a tube containing a small amount of potassium oxalate. The samples were placed in ice water and protected from light immediately after being withdrawn. In no instance was the blood allowed to stand at room temperature. The plasma filtrates were always prepared within two hours from the time the samples were taken. The method used for the determination of ascorbic acid was that described by Mindlin and Butler (4), with a slight modification.

In order to observe any variations in the concentration of ascorbic acid due to the method of determination and also to determine the stability of the ascorbic acid in the prepared filtrate, duplicate filtrates were prepared from the blood of five cows and stored at 2° C. The ascorbic acid was determined in the stored filtrates at 3-hour intervals for a 24-hour period (table 5).

RESULTS

The experimental data showing the variations in the concentration of ascorbic acid in the plasma of 19 normal calves from birth to 12 months of age are presented in table 1. The mean value obtained from all of the determinations was 0.32 mg. per 100 ml. of plasma but the variations covered a wide range of values (0.03 to 0.77 mg.). The mean values for individual calves also showed considerable variation, ranging from 0.09 to 0.56 mg., however the calves under eight weeks of age had a tendency to have less ascorbic acid in the plasma (mean 0.30 mg., range 0.03 to 0.55 mg.) than the older calves (mean 0.36 mg., range 0.16 to 0.77 mg.).

The variations in the concentration of ascorbic acid in the plasma of the four heifers are shown also in table 1. The mean value obtained from all of the determinations was 0.49 mg. per 100 ml. of plasma (range 0.24 to 0.80 mg.). The mean values obtained for the individual heifers varied from 0.36 to 0.63 mg.

TABLE 1

Plasma ascorbic acid values obtained from normal calves and heifers

Calf No.	Breed	Ave. age	No. samples taken	Range	Mean
			Calves		
				mg. per 100 ml. of plasma	
C495	Ayrshire	3 wk.	3	0.18–0.35	0.27
C466	Holstein	4 wk.	2	0.41–0.48	0.44
C468	Holstein	4 wk.	2	0.38–0.52	0.45
C483	Holstein	4 wk.	4	0.16–0.38	0.28
C498	Holstein	4 wk.	5	0.16–0.29	0.25
C461	Guernsey	4 wk.	8	0.05–0.26	0.17
C463	Guernsey	4 wk.	6	0.13–0.53	0.38
C464	Guernsey	4 wk.	6	0.21–0.38	0.30
C496	Jersey	5 wk.	6	0.16–0.42	0.25
C462	Holstein	6 wk.	9	0.21–0.51	0.36
C459	Brown Swiss	7 wk.	2	0.03–0.16	0.09
C460	Holstein	7 wk.	7	0.09–0.31	0.17
C465	Jersey	7 wk.	7	0.34–0.55	0.46
C452	Holstein	5 mo.	4	0.16–0.36	0.24
C456	Holstein	9 mo.	9	0.21–0.34	0.28
C457	Holstein	9 mo.	9	0.18–0.31	0.24
A35	Holstein	9 mo.	10	0.25–0.67	0.36
A34	Holstein	11 mo.	7	0.44–0.77	0.56
A33	Holstein	12 mo.	10	0.38–0.64	0.50
Mean				0.03–0.77	0.32
			Heifers		
A31	Holstein	20 mo.	9	0.42–0.62	0.54
C424	Holstein	28 mo.	9	0.24–0.49	0.36
16	Guernsey	29 mo.	9	0.26–0.59	0.42
A29	Holstein	34 mo.	9	0.46–0.80	0.63
Mean				0.24–0.80	0.49

The mean and range of ascorbic acid values obtained for the 24 mature cows, either open or in various stages of pregnancy, are presented in table 2. The mean values obtained for the individual cows varied between 0.29 and 0.65 mg. per 100 ml. of plasma, whereas the mean of all of the values was 0.44 mg. (range 0.11 to 0.80 mg.). There was no significant difference in the means or range of values between the pregnant and open cows although the ascorbic acid values had a tendency to be higher in the blood of the pregnant cows.

Table 3 shows the variations found at weekly intervals in the concentration of ascorbic acid in the plasma of six calves from birth to 10 weeks of age on a whole milk ration. The mean value obtained for this group of

TABLE 2

Plasma ascorbic acid values obtained from normal cows

Cow No.	Breed	Age	No. samples taken	Range	Mean
		yr.		*mg per 100 ml. of plasma*	
66	Jersey	3	9	0.17–0.46	0.33
289	Holstein	3	20	0.20–0.61	0.37
290	Holstein	3	8	0.25–0.58	0.47
A26	Holstein	3	22	0.40–0.80	0.61
A27	Holstein	3	27	0.48–0.78	0.63
A25	Holstein	4	9	0.48–0.77	0.58
284	Holstein	4	7	0.26–0.63	0.40
285	Holstein	4	20	0.23–0.53	0.36
A22	Holstein	5	27	0.24–0.55	0.39
A23	Holstein	5	19	0.31–0.61	0.41
269	Holstein	5	20	0.28–0.55	0.39
A18	Holstein	6	17	0.38–0.72	0.54
78	Jersey	6	9	0.36–0.53	0.43
264	Holstein	6	17	0.21–0.55	0.39
266	Holstein	6	18	0.40–0.61	0.50
267	Holstein	6	17	0.43–0.53	0.46
A15	Holstein	7	2	0.27–0.52	0.39
237	Brown Swiss	7	9	0.53–0.76	0.65
A14	Holstein	8	9	0.29–0.43	0.35
239	Brown Swiss	8	9	0.42–0.57	0.46
A6	Holstein	10	23	0.11–0.46	0.30
D14	Holstein	13	9	0.24–0.36	0.29
D5	Holstein	14	18	0.20–0.71	0.37
D9	Holstein	14	11	0.39–0.53	0.40
Mean				0.11–0.80	0.44

TABLE 3

Variations in the concentration of plasma ascorbic acid in young calves and cows

Calf No.	Age in weeks										Mean
	1	2	3	4	5	6	7	8	9	10	
					Calves						
					mg. per 100 ml. of plasma						
C460				0.14	0.15	0.17	0.09	0.31	0.11	0.21	0.17
C461	0.05	0.19	0.14	0.25	0.12	0.12	0.26	0.21			0.17
C462		0.43	0.26	0.21	0.42	0.35	0.28	0.51	0.29	0.47	0.36
C463	0.45	0.28	0.53	0.50		0.40		0.13			0.38
C464		0.26	0.33	0.34	0.21	0.28	0.38				0.30
C465				0.34	0.43	0.54	0.47	0.42	0.44	0.55	0.46
Mean											0.31
					Cows*						
269	0.29	0.55	0.52	0.40	0.38	0.32	0.28	0.38	0.43	0.42	0.40
285	0.26	0.23	0.48	0.23	0.53	0.35	0.30	0.37	0.32	0.38	0.35
289	0.38	0.48	0.31	0.61	0.31	0.30	0.38	0.32	0.20	0.47	0.38
290	0.29	0.25	0.58	0.54	0.58	0.58	0.37	0.57			0.47
A26	0.47	0.58	0.51	0.58	0.58						0.54
A27	0.74	0.54	0.61	0.54	0.48	0.67	0.58	0.57	0.61		0.59
Mean											0.46

* Samples taken at irregular intervals over a period of six months.

calves was 0.31 mg. per 100 ml. of plasma (range 0.05 to 0.55 mg.). The outstanding feature of table 3 is the marked variations that occurred in the concentration of ascorbic acid from week to week. The very low values obtained for calf C460 were not associated with malnutrition or disease. The calf was normal in all respects. Calf C461, on the other hand, had very low ascorbic acid values and died from gastro-enteritis but calf C463 had fairly high ascorbic acid values and died from gastro-enteritis also. It is not likely that the amount of ascorbic acid in the blood plasma had any relationship to the disease. The other three calves were in good condition and in normal health during the time these samples were taken.

The individual mean and range of ascorbic acid values obtained at irregular intervals over a period of six months for six lactating Holstein cows are presented in table 3. The individual mean values varied from 0.35 to 0.59 mg. per 100 ml. of plasma but the mean value obtained for this group of cows was about the same as that given in table 2.

TABLE 4

Daily variation in the amount of plasma ascorbic acid in lactating Holstein cows

Animal No.	1	2	3	4	5	6	7	8	9	10	Mean
	mg. per 100 ml. of plasma										
A6	0.33	0.43	0.45	0.29	0.23	0.28	0.23	0.17	0.25	0.26	0.29
A23	0.59	0.59	0.51	0.37	0.48	0.44	0.28	0.19	0.22	0.16	0.38
269	0.52	0.53	0.40	0.32	0.33	0.38	0.38	0.24	0.32	0.28	0.37
285	0.53	0.55	0.40	0.35	0.35	0.37	0.30	0.37	0.29	0.37	0.39
289	0.61	0.50	0.31	0.40	0.30	0.36	0.38	0.19	0.32	0.31	0.37
A18	0.56	0.59	0.59	0.56	0.73	0.70	0.50	0.56			0.60
267	0.40	0.43	0.33	0.42	0.55	0.53	0.48	0.42			0.45
D9	0.36	0.33	0.31	0.35	0.53	0.42	0.44	0.26			0.38
Mean											0.41

Table 4 shows the daily variations that occurred in plasma ascorbic acid of eight lactating Holstein cows. The cows were bled each day at the same hour for a period of 8 or 10 days. Considerable individual variation occurred from day to day in all of the cows but the widest range was shown by cows A23 and 289 and the least variation was shown by cows A18 and 267. The values presented in table 4 indicate that there may be as much variation in plasma ascorbic acid from day to day as there is from animal to animal.

The data in table 5 show the diurnal variations that occurred in the plasma of heifers and lactating cows during a 24-hour period. The diurnal variations were determined for five cows (A22, A26, A27, 264 and 266) for two different 24-hour periods. Considerable variation was found for many 3-hour periods in all of these cows but these variations could not be correlated with the time of feeding or the time after feeding. The mean daily values observed for A26, A27 and 266 for two different 24-hour periods were not significantly different. Cow A22 had a mean ascorbic acid value of 0.31

mg. per 100 ml. of plasma for one 24-hour period and 0.43 mg. for the other 24-hour period. Cow 264 had a mean value of 0.30 mg. for one 24-hour period and 0.47 mg. for the other period.

The narrowest range observed for any one 24-hour period was for cow 267 (minimum 0.43 mg., maximum 0.53 mg.) and the widest range was observed in cow A26 (minimum 0.40 mg., maximum 0.80 mg.). When the differences between the minimum and maximum values obtained at 3-hour intervals were summarized for each 24-hour period it was found that 5 cows had differences between 0.10 and 0.20 mg., 8 cows had differences between 0.21 and 0.30 mg., and only 2 cows showed differences between 0.31 and 0.40 mg. The major differences were not observed to occur at any regular interval during the day. When ascorbic acid determinations were made for two cows at 6-hour intervals for a 48-hour period, it was found that the range of values was similar to that obtained at 3-hour intervals and that the minimum and maximum values did not occur at the same time each day.

TABLE 5

Diurnal variations in the concentration of plasma ascorbic acid in dairy cattle

Animal No.	Three hour interval									Mean
	1	2	3	4	5	6	7	8	9	
	mg. per 100 ml. plasma									
237*	0.70	0.76	0.59	0.53	0.62	0.60	0.69	0.76	0.61	0.65
237†	0.70	0.70	0.70	0.70	0.64	0.70	0.70	0.70	0.73	0.70
264*	0.52	0.53	0.34	0.45	0.48	0.44	0.50	0.55	0.45	0.47
264†	0.52	0.53	0.52	0.47	0.54	0.51	0.51	0.51	0.56	0.52
264‡	0.43	0.39	0.23	0.35	0.29	0.27	0.21	0.23		0.30
A22*	0.40	0.37	0.29	0.44	0.54	0.38	0.44	0.55	0.48	0.43
A22†	0.40	0.40	0.40	0.40	0.48	0.39	0.43	0.43	0.43	0.42
A22‡	0.31	0.36	0.31	0.29	0.29	0.35	0.33	0.34	0.24	0.31
A25*	0.48	0.51	0.77	0.63	0.62	0.61	0.62	0.51	0.48	0.58
A25†	0.51	0.50	0.50	0.51	0.51	0.51	0.51	0.47	0.48	0.50
266*	0.40	0.44	0.61	0.52	0.40	0.48	0.54	0.50	0.51	0.49
266†	0.42	0.40	0.40	0.42	0.42	0.42	0.42	0.39	0.39	0.41
266‡	0.57	0.57	0.58	0.50	0.49	0.47	0.47	0.56	0.47	0.52
267	0.53	0.46	0.43	0.46	0.43	0.50	0.52	0.46		0.47
66	0.46	0.37	0.37	0.24	0.25	0.38	0.40	0.32	0.17	0.33
78	0.50	0.52	0.53	0.43	0.36	0.36	0.36	0.43	0.36	0.43
239	0.50	0.57	0.50	0.43	0.47	0.43	0.42	0.43	0.43	0.46
A14	0.35	0.36	0.36	0.43	0.36	0.36	0.31	0.33	0.29	0.35
A18	0.62	0.38	0.38	0.72	0.39	0.43	0.50	0.52		0.49
A26‡	0.67	0.60	0.66	0.62	0.66	0.59	0.60	0.71		0.64
A26‡	0.72	0.64	0.65	0.62	0.66	0.80	0.60	0.51	0.40	0.62
A27‡	0.77	0.70	0.69	0.62	0.61	0.78	0.64	0.57	0.70	0.68
A27‡	0.67	0.60	0.68	0.54	0.70	0.72	0.49	0.48	0.58	0.61
C424	0.49	0.43	0.36	0.29	0.24	0.31	0.35	0.35	0.29	0.35
D14	0.28	0.29	0.26	0.24	0.26	0.33	0.28	0.31	0.36	0.29

* Filtrate prepared from blood sample taken at 3 hour interval.

† Filtrate prepared at start of experiment from plasma of first sample and analyzed at 3-hour intervals.

‡ Samples obtained on different dates.

The data presented in table 5 also indicate that plasma filtrates can be stored at 2° C. for 24 hours without any significant loss of ascorbic acid. The variations in the plasma filtrate which did occur in the determinations made at 3-hour intervals were within the accuracy of the method and could not be ascribed to changes in concentration of ascorbic acid. The maximum variations that occurred above and below the initial values were + 0.08 mg. and – 0.06 mg. per 100 ml. of plasma. These variations were usually less than those obtained from the blood samples taken at 3-hour intervals. The major variations obtained in the samples taken at 3-hour intervals are interpreted to mean actual differences in the amount of ascorbic acid in the plasma and not due to the method of determination.

DISCUSSION

The results of this investigation indicate very clearly that there are marked differences in the amount of ascorbic acid in the plasma of normal calves and lactating cows. The results also indicate that there are even greater differences in the level of ascorbic acid in the plasma of individual normal animals. A value that might be considered normal for one animal could be considerably higher or lower than another normal animal of the same age or same physiological condition. The values were found to vary not only from animal to animal but also major variations were found to occur from day to day and from hour to hour.

The mean and range of ascorbic acid values obtained from 356 determinations on 24 normal cows was found to be 0.44 mg. per 100 ml. of plasma (range 0.11 to 0.80 mg.). These values are in agreement with the values reported by Phillips *et al.* (6) but they are considerably lower than the range of values reported by Knight and co-workers (3) and they are higher than the mean value reported by Wallis (11).

From the results of this investigation it would appear that the normal level of ascorbic acid cannot be determined with any degree of accuracy by random sampling. Wide fluctuations were found to occur from day to day and from one 3-hour period to another under presumably identical conditions. These fluctuations could not be correlated with the normal period of feeding even though many of the blood samples were taken at the same hour each day.

The average content of ascorbic acid in the blood of calves from birth to 12 months of age was 0.32 mg. per 100 ml. of plasma whereas the ascorbic acid in the blood of heifers from 20 to 34 months of age was 0.49 mg. The number of animals used in this study was too small to make the results conclusive but they indicate that the ascorbic acid content of the blood of young calves is low and that there is a tendency for this vitamin to increase as the animal reaches maturity.

ing part of the time, composite weekly samples of milk were used in determining the fat percentages from which the monthly yields of butterfat were computed. During the balance of the time, the fat percentages were determined from the composite sample (sometimes calculated from the fat determinations of each milking) of only one day's milk yield each month. All records were used as actually made without any corrections for age, etc.

Dairy herd improvement association material. The large proportion of the 1289 lactations which came from five breeds (grades and purebreds) were made by Holsteins (554 lactations), Guernseys (422 lactations) and Jerseys (199 lactations). Only the first ten months of each lactation were used. Some of the lactations were shorter than this though no record that was not a complete lactation was used.

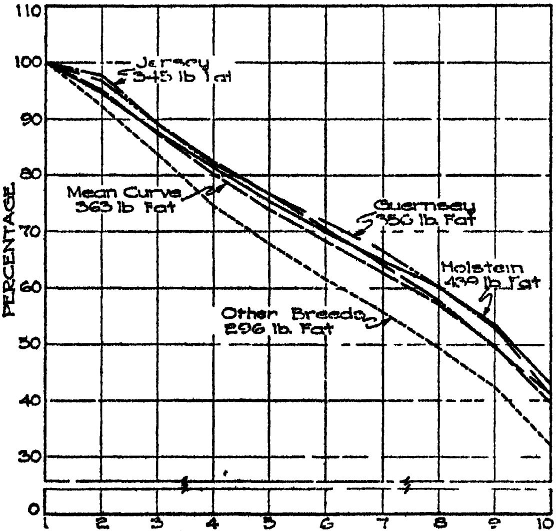

FIG. 1. Curves showing the perentage relationship of each month's yield to that of the first month for cows in the Iowa State College herd.

In Dairy Herd Improvement Association testing, a single day's milk yield and its butterfat percentage is obtained from which the month's yield is calculated. Since each month's yield is a multiple of its day's yield, calculations of the lactation curves on a percentage basis would be the same when based on the day's yield as when based on the month's yield. Factors of prediction determined from the days' yields also would have the same relation to each other as factors determined from the months' yields.

In Iowa, each D.H.I.A. supervisor is required to record originally milk yield and butterfat percentage for each cow in a herd, breeding and calving dates, and unusual conditions affecting the cow's production on a "barn sheet," carbon copies of which are forwarded to the state supervisor's office each month. These supplied the data for this investigation.

As in the case of the college records all Dairy Herd Improvement Association records were used without any correction, though the records were made by cows at all ages.

RESULTS AND DISCUSSION

Although only the Holstein-Friesian records made in the college herd were used for the calculation of equations for predicting yield (regression equations) the records of other breeds in the herd were analyzed to determine if there were marked breed differences in the slope of the lactation curves.

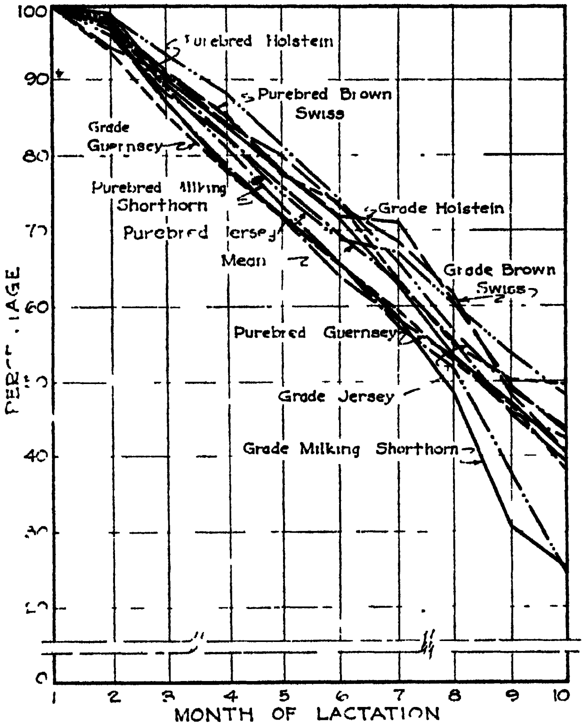

FIG. 2. Curves showing the percentage relationship of each month's yield to that of the first month for cows of different breeds in the D.H.I.A.

Figure 1, shows a great similarity between the curves of the Guernseys, Holsteins and Jerseys. The other breeds failed to show the same persistency but the records involved were so few their curves may not be typical.

The same similarity in the lactation curves of the different breeds was shown by the records made in the Dairy Herd Improvement Association (fig. 2). Because of this no segregation of records by breeds was made in the calculation of prediction factors (regression equation) from the Dairy Herd Improvement Association data.

The essential items obtained in the statistical analyses of both sets of data are presented in tables 1 and 2.

TABLE 1

Data on 400 Holstein-Friesian lactations showing the linear regression coefficient, regression equation, standard error of estimate and correlation

(Iowa State College herd data)

Month of lactation	No. of records	$\bar{x}$	σx	$\bar{y}$	σy	Regression equation*	Standard error of estimate	Correlation coefficient
1–A	400	50.4	12.5	369.1	108	4.99A + 117	73.2	0.74
2–B	400	47.8	14.2	369.1	108	6.02B + 81	66.6	0.79
3–C	400	44.3	12.9	369.1	108	7.19C + 51	56.1	0.86
4–D	400	41.2	12.6	369.1	108	7.53D + 59	51.6	0.88
5–E	398	38.3	11.7	370.1	107	8.40E + 49	43.8	0.91
6–F	397	35.6	11.5	370.5	107	8.50F + 68	44.4	0.91
7–G	390	33.7	11.2	372.4	107	8.55G + 84	46.1	0.90
8–H	388	31.4	11.2	373.1	107	8.28H + 113	52.1	0.87
9–I	374	28.8	11.8	377.1	105	7.01I + 175	65.2	0.79
10–J	350	24.8	12.7	381.1	106	5.61J + 242	78.9	0.67

* When A equals fat yield of first month, B equals fat yield of second month, etc.

TABLE 2

Data on 1289 lactations showing the linear regression coefficient, regression equation, standard error of estimate and correlation (D.H.I.A. records)

Month of lactation	No. of records	$\bar{x}$	σx	$\bar{y}$	σy	Regression equations*	Standard error of estimate	Correlation coefficient
1–A	1289	1.64	0.47	11.17	2.82	4 08A + 135	61.5	0.40
2–B	1289	1.52	0.43	11.17	2.82	5.13B + 101	51.9	0.50
3–C	1289	1.40	0.39	11.17	2.82	6.00C + 83	47.7	0.59
4–D	1289	1.30	0.36	11.17	2.82	6.41D + 85	48.0	0.63
5–E	1289	1.21	0.34	11.17	2.82	5.06E + 152	45.6	0.69
6–F	1289	1.12	0.32	11.17	2.82	5.55F + 149	46.8	0.72
7–G	1287	1.02	0.33	11.18	2.80	6.55G + 135	48.0	0.70
8–H	1245	0.91	0.33	11.29	2.78	7.18H + 143	56.7	0.64
9–I	1093	0.79	0.35	11.56	2.89	7.25I + 175	73.5	0.48
10–J	791	0.70	0.30	11.92	2.79	6.43J + 223	74.7	0.68

* When A equals fat yield of the test day in first month, B equals fat yield of the test day in second month, etc.

Table 3 shows the decline in yield from month to month, of the cows in the college herd and in the Dairy Herd Improvement Associations. These figures calculated as percentages of the first month's yield and as percentages of the previous month's yield indicate some differences in persistency between the two groups. The persistency index of the College cows was 92.3 and for the D.H.I.A. cows 91.0 when calculated according to Turner's method (4).

TABLE 3

Month to month decline in yield of College and D.H.I.A. cows calculated as percentage of the first month yield and as percentage of the previous month's yield

Month of test	College data			D.H.I.A. data		
	Yield	Per cent of 1st month	Per cent of previous month	Yield	Per cent of 1st month	Per cent of previous month
1	50.4	100.0		1.64	100.0	
2	47.8	94.8	94.8	1.52	92.7	92.7
3	44.3	87.8	92.7	1.40	85.4	92.1
4	41.2	81.7	93.0	1.30	79.3	92.9
5	38.2	75.8	92.7	1.21	73.8	93.1
6	35.6	70.6	93.2	1.12	68.3	92.6
7	33.7	66.8	94.7	1.02	62.2	91.1
8	31.4	62.3	93.2	0.91	55.5	89.2
9	28.8	57.1	91.7	0.79	48.2	86.8
10	24.8	49.2	86.1	0.70	42.7	88.6
	Persistency index 92.3			Persistency index 91.0		

The prediction of yield according to our results is most accurately made from a test taken during the fifth month of lactation as shown by the low standard error of estimate. This is true both with records obtained from the College herd and from D.H.I.A. herds (tables 1 and 2). The tests made in the sixth month gave the next most accurate predictions, followed in turn by those made in the seventh and fourth months. These results varied somewhat from those obtained by Gaines (1) and Kartha (3) who found that a short-time test taken in the fourth month after calving gave the most accurate basis for estimating a 305-day record. There is less accuracy in predicting yield when based on tests made in the first or last months of the 10-months lactation than there is when based on tests made nearer the middle of the lactation.

Sometimes one wishes to estimate what a cow might produce when only a part of the lactation record is known. Occasionally a cow will die or become seriously injured during a lactation and her 10-month record is wanted in the proving of her sire. Factors for these estimates were made from our data. They are found in table 4 and are for estimating the ten months yield of butterfat.

Dairy men may use the regression equations in tables 1 and 2 as factors for predicting 10-month yields of dairy cows when only one day's yield is known. The day's yield of butterfat is multiplied by 30.5, the average number of days in a month, to obtain the month's yield. The elapsed time between 22 days after the day of calving[1] and the day on which the yield is taken will determine the month of lactation which will also determine the factor to use in making the estimation of yield.

In table 5 are records of cows selected at random from four herds in Iowa Dairy Herd Improvement Associations. Variations occur between the estimated yields (columns 9 and 10) and the actual yields (column 11) which are sometimes rather large (cow 12) while other estimates are very close to the actual yield (cow 3). It is not expected that the estimated yield for every single cow will be as close to the actual as is the case with cow 3.

TABLE 4

Factors for correcting partial lactations to ten months lactations (butterfat yield basis)*

Months in milk	Factors based on data collected from	
	College herd	D.H.I.A. herds
1	7.47	7.08
2	3.83	3.67
3	2.64	2.55
4	2.05	1.98
5	1.70	1.64
6	1.46	1.42
7	1.29	1.26
8	1.17	1.15
9	1.07	1.06

* Multiply the total yield for the number of months in milk by the corresponding factor.

If a few cows are used then the variations in one direction tend to cancel out those in the other. In the case of the fifteen cows used in table 5 the total estimated yield for all the cows is 132 pounds or an average per cow of 8.8 pounds of fat greater than the actual yield when the "College" factors are used and 46 pounds or an average of 3.0 pounds of fat less when the "D.H.I.A." factors are used.

It would be an unusual person who could estimate the probable yield of these fifteen cows as closely as this from a mere physical examination of them. The little extra effort of taking a single day's milk yield and butterfat test on each cow permits knowing rather accurately what to expect in the way of production from the lactations the cows are passing through. The physical examination and the test with the accompanying estimated yields give a much greater basis for judgment than either alone.

Because the regression coefficients and the factors for correcting partial lactations calculated from the College herd data (tables 1 and 4) were from

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[1] There is a lag of 22 days from calving until the first testing period in D.H.I.A. records.

TABLE 5

Estimated yields (fat) of unselected cows calculated from factors derived from College and D.H.I.A. data compared with actual yields taken from the D.H.I.A. records

1 Cow No.	2 Breed	3 Date of freshening M-D-Y	4 Date of test M-D-Y	5 Months in lactation*	6 Day's milk (lbs.)	7 Production fat	8	9 Estimated 305-day fat yields, in lbs, using	10	11 Actual 305-day fat yields in lbs. according D.H.I.A.
						Per cent	Lbs.	College factors	D.H.I.A. factors	
1	G.H.	8- 3-40	4-21-41	8	27.8	3.1	0.86	330	331	310
2	G.H.	3- 3-41	4-21-41	1	66.3	3.0	1.99	420	383	424
3	G.H.	2- 1-41	4-21-41	2	38.2	3.0	1.14	290	279	283
4	R.H.	11-30-40	4-21-41	4	39.4	3.1	1.22	339	323	310
5	G.H.	1- 2-41	4-21-41	3	50.1	3.5	1.75	434	403	437
6	G.J.	1-19-41	3- 2-41	1	33.1	6.3	2.08	433	394	452
7	G.G.	10-25-40	8-18-41	10	16.6	6.5	1.07	425	433	493
8	G.G.	11-20-40	8-18-41	9	19.8	4.8	0.95	378	385	443
9	G.G.	10-29-40	8-18-41	9	17.2	4.3	0.73	331	336	297
10	G.G.	11- 1-40	8-18-41	9	16.8	5.5	0.92	372	379	346
11	G.H.	9-22-40	5- 6-41	7	31.9	2.9	0.93	327	321	356
12	R.H.	10-28-40	5- 6-41	6	41.5	3.8	1.58	478	417	372
13	G.H.	12-27-40	5- 6-41	4	37.2	2.9	1.08	307	280	219
14	R.H.	9-17-40	5- 6-41	7	24.8	2.7	0.67	258	269	293
15	-- --	10-12-40	5- 6-41	6	24.5	3.2	0.78	270	281	225
						Total		5392	5214	5260
						Total error		+ 132	- 46	
						Aver. error		+ 8.8 lbs. per cow	- 3.0 lbs. per cow	

* An easy way to calculate the "months in lactation" is to subtract the date of freshening from the date of test as

Year — Month — Day
40 ~~41~~ 16 ~~4~~ 21 This indicates that 8 months and 18 days
40 8 3 have elapsed
— — —
8 18

between the date of calving and the date of test. Since there is a lag of 22 days the factor to be used should be that for the 8th month. Had the number of days beyond 8 months exceeded 22 days then the factor for the 9th month would have been used.

records made by Holstein cows only, their use should not be as generally applied as those calculated from the Dairy Herd Improvement Association data (tables 2 and 4). These latter were calculated from records made under more general conditions so their use would give more conservative results and would better fit the conditions of most farmers' herds.

In reality the variations in records calculated from the two sets of factors are not great and either can be used successfully.

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GRAPHIC METHOD SHOWING EFFICIENCY OF DAIRY BULLS

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Dairy bulls are commonly evaluated by comparing the yields of daughters and dams. The result is usually expressed as an index which is supposed to represent an estimate of what the production of the bull's daughters would be if they were not influenced by the hereditary effect of their dams.

Such an index is concise and valuable but for educational purposes in classes or farmers' meetings it may be helpful to illustrate the efficiency of bulls by a graphic method such as that used in the accompanying chart.

In this chart the butterfat production of dams and daughters from six different bulls is shown. The records are from a small dairy herd over a period of years.

The graphs are so designed that when the records of a dam and daughter are equal, the dot will fall on the diagonal line; if the daughter's production is greater than the dam's the dot will be above, while if it is less than the dam's it will be beneath the diagonal line. By noticing the position of the dots one can tell at a glance whether the bull is increasing or decreasing the production of the herd.

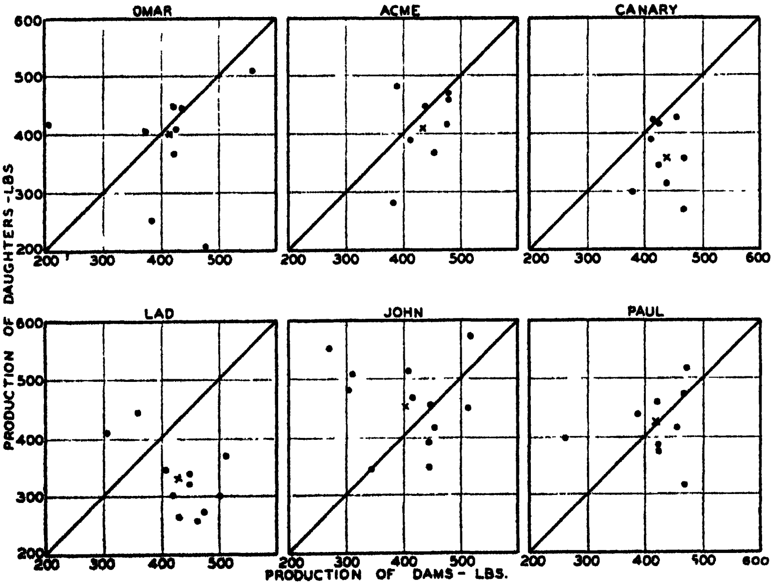

FIG. 1. Effect of bulls on production in a dairy herd. Each dot represents the production of a dam and her daughter. X represents the average of dams and daughters.

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Note for instance, that in the Canary and Lad diagrams the dots are almost entirely beneath the diagonal line and indicate the extent to which the daughters were inferior to their dams. On the other hand, John was a superior bull with eight out of twelve daughters equal to or superior to their dams.

The average production of any group of dams and daughters is indicated by the position of the X within each major square representing any given bull.

The position of the X indicates that of the six dairy bulls, only John and Paul increased the average productivity of the herd. Canary and Lad were inferior bulls that reduced the average production of the herd to a remarkable degree. It is interesting to note that Canary was a show bull that had won some ribbons at fairs but his value lay chiefly in his looks, not in his performance.

The reliability of the average production of the daughters from each bull can be estimated by the compactness of grouping of the dots.

THE ADDITION OF CAROTENOIDS AS A MEANS OF MAINTAINING A UNIFORM COLOR IN MILK AS MEASURED BY THE LAC-CHROM-METER

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For a long time milk consumers have noticed that the milk which they buy varies in color throughout the year. Milk normally has a deeper yellow color during the spring and summer when cows have access to fresh green pasture.

In this investigation color determinations of milk of the five breeds, Ayrshire, Guernsey, Holstein, Jersey and Brown Swiss were made using a Munsell "Lac-chrom-meter."[2] Figure 1 shows a diagrammatical sketch of the instrument used. The procedure for making the color determination is briefly as follows: the instrument is properly balanced, the sample cell C is filled with the milk to be tested, placed on the sample shelf SH, and the light B moved until the spot S in the center of the field disappears. The point where the pointer shadow falls on the scale indicates the color grade of the sample measured. Garrett *et al.* (3) have given the principles involved in the construction and operation of a similar instrument known as a "lactochrometer."

The effect of the direct addition of carotene on the color of the milk was also measured with the same instrument in an effort to determine the feasibility of standardizing the color of milk throughout the year by the direct addition of carotene during seasons when the normal color of the milk is low. At various intervals carotene determinations on the normal milk were made in an effort to find out if there was any correlation between the color of the milk as determined on the "Lac-chrom-meter" and the carotene content of the same milk.

Baumann and Steenbock (2) found that "carotene addition to winter butter in an attempt to increase the vitamin A activity up to that of summer butter does not appear to be practical unless the public should be found to be willing to accept a much more highly colored product than at present."

The milk from the various breeds was collected from four or more cows of the same breed in the Ohio State University dairy herd. The cows were stabled from the middle of November until April 21 at which time they were

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[1] Now located at Colorado State College, Fort Collins, Colorado.

[2] The instrument used was loaned through the courtesy of the American Guernsey Cattle Club.

turned out on pasture. During the period from February 17 to April 21, they received 24 to 36 pounds of molasses alfalfa silage, 7 to 12 pounds of grain and all the good quality leafy green alfalfa hay they would eat. The mixed herd samples, which were tested, were taken from the University herd mixed-milk after it had been delivered to the University dairy and dumped into the weigh tank. By using such milk, a representative mixed milk could be obtained as the University herd is made up of cows from the five breeds named above. The homogenized milk used was the same mixed milk after it had been homogenized and pasteurized.

When readings were made on the various milks standardized to five per cent fat, a portion of the milk was separated by gravity and the cream and skimmilk recombined in the amounts necessary to produce a milk testing five per cent.

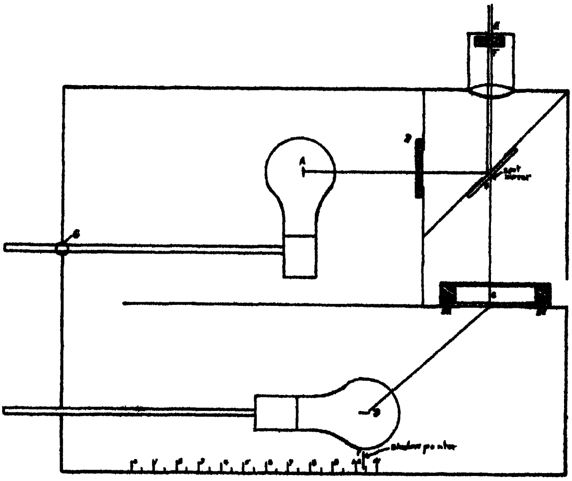

FIG. 1. Diagrammatical sketch of the "Lac-chrom meter."

The source of carotene used was "Provalac," a commercial beta carotene concentrate emulsified in butterfat, furnished by the General Biochemicals Company, of Chagrin Falls, Ohio. It was diluted by mixing one part of the concentrate with nine parts of homogenized milk. This solution was then pipetted into the samples at the rate necessary to give an addition of 300, 600 and 900 micrograms carotene per quart, equal to 500, 1000 and 1500 International Units of vitamin A, respectively.

For the carotene determinations, a portion of the natural milk was separated by gravity, the cream being churned into butter which was analyzed for carotene content spectrophotometrically at the Ohio Agricultural Experiment Station.

The results of these experiments are given in table 1. According to the "Lac-chrom-meter" readings, Guernsey milk was consistently higher in color than that of the other breeds, followed in order by Jersey, Ayrshire and Brown Swiss (the latter two being approximately the same), and Holstein which showed the least color.

From February 17 until April 21, at which time the cows were turned on pasture, the color of the milk within the various breeds varied to some extent but was generally lower than the color exhibited by the same breed after the cows were turned on pasture. This increase in color is attributed to the increased carotene content of the milk due to the cows being placed on green pasture. The milk of the Jersey and Guernsey breeds increased in color to a greater extent than that of the other breeds. The results indicate that the milk of each breed reached its peak in color approximately three weeks after the cows were turned on pasture, and after the peak was reached, there was a slow but general decline in color into August for some of the breeds, yet the carotene content of the milk actually increased as shown in table 1.

The results presented in table 1 show that by adding carotene to the milk of the various breeds or to mixed herd milk, the color of the milk is increased by approximately 1.0 "Lac-chrom-meter" unit with each addition of 300 micrograms of carotene. On this basis it would have required the addition of about 450 micrograms of carotene per quart to the winter milk to bring about a color somewhere near the maximum obtained when the cows were on pasture.

Since the color of milk may be increased by adding carotene directly to the milk, visual comparisons of the various types of milk used in this experiment were made using normal Guernsey milk as a standard. Samples of the various types of milk were placed in test tubes of the same diameter and carotene was added to them until they matched the color of the normal Guernsey milk according to a visual comparison. Carotene in the following amounts per quart of five per cent milk was needed to match the Guernsey milk in most instances: 600 micrograms for Ayrshire, Holstein, and Brown Swiss; 180 for Jersey; 450 for mixed herd milk; and 300 for the same mixed herd milk after it was homogenized. The color of the milk to which carotene was added did not exactly match the color of the Guernsey milk, as it had somewhat of a reddish tinge, slightly different from the normal yellow color of the Guernsey milk. The visual color seemed to increase to a greater extent on the addition of carotene than did the "Lac-chrom-meter" units.

The comparison between the "Lac-chrom-meter" reading and carotene content shows only a general correlation in this respect and not a direct one, as an inspection of table 1 will show. This indicates that the "Lac-chrom-meter" reading is influenced by other factors than the carotene content of the milk. That there is a correlation between the fat content and color of milk has been shown by Bartlett *et al.* (1). The fat content of the milk affected the "Lac-chrom-meter" reading. This point is illustrated in table

TABLE 1

Color determinations as made on the Munsell "Lac-chrom-meter."
Results expressed as Lac-chrom-meter units

Raw Ayrshire Milk

Date	Normal milk			Normal milk with carotene added (Lac-chrom-meter units)			Standardized to 5% fat	Micrograms of carotene per quart to bring visual color of milk to that of Guernsey (both milks standardized to 5% fat)
	Fat content	Lac-chrom-meter units	Carotene content (mg./kg. fat)	300 µg.	600 µg.	900 µg.	(Lac-chrom-meter units)	
2-17-41	......	1.0	......	2.2	3.3	4.4	......	......
2-25-41	4.1	1.1	1.04	2.2	3.3	4.3	......	......
3-13-41	4.1	1.8	......	2.9	4.0	5.0	......	......
3-20-41	4.3	1.1	......	2.1	3.1	4.1	......	......
4-14-41	3.8	1.1	......	2.1	3.1	4.1	......	......
4-20-41	3.7	1.0	1.50	2.0	4.1	4.1	......	......
5- 7-41	3.9	2.0	3.08	3.0	4.0	5.0	......	......
5-14-41	4.3	2.4	......	3.4	4.4	5.4	2.9	600
5-27-41	4.0	1.9	......	2.8	3.9	4.9	3.0	600
6- 2-41	3.9	1.8	......	2.7	3.7	4.8	3.5	600
6-17-41	4.0	1.5	......	2.4	3.4	4.4	2.9	600
7-28-41	4.0	1.4	3.65	2.3	3.4	4.4	2.8	600

TABLE 1.—(*Continued*)

Raw Brown Swiss Milk

Date	Normal milk			Normal milk with carotene added (Lac-chrom-meter units)			Standardized to 5% fat	Micrograms of carotene per quart to bring visual color of milk to that of Guernsey (both milks standardized to 5% fat)
	Fat content	Lac-chrom-meter units	Carotene content (mg./kg. fat)	300 µg.	600 µg.	900 µg.	(Lac-chrom-meter units)	
2–17–41		0.9	...	2.0	3.0	4.1	...	...
2–25–41	4.0	0.6	1.58	1.6	2.5	3.6	...	...
3–13–41	4.3	1.9	...	3.0	4.0	5.0	...	...
3–29–41	4.2	1.1	...	2.0	3.2	4.2	...	...
4–14–41	3.5	0.6		1.6	2.6	3.6	...	...
4–20–41	3.5	0.5	1.92	1.6	2.6	3.5	...	...
5– 7–41	3.7	1.6	4.40	2.5	3.4	4.3		...
5–14–41	4.5	2.5	...	3.6	4.5	5.5	2.8	600
5–27–41	4.0	1.8	...	2.7	3.8	4.8	3.0	600
6– 2–41	4.0	1.8	...	2.7	3.7	4.6	3.6	600
6–17–41	3.7	1.5	...	2.5	3.4	4.4	3.3	600
7–28–41	3.8	1.4	4.20	2.4	3.4	4.4	3.2	600

TABLE 1.—(*Continued*)

Raw Guernsey Milk

Date	Normal milk			Normal milk with carotene added (Lac-chrom meter units)			Standardized to 5% fat	Micrograms of carotene per quart to bring visual color of milk to that of Guernsey (both milks standardized to 5% fat)
	Fat content	Lac chrom-meter units	Carotene content (mg./kg. fat)	300 µg.	600 µg.	900 µg.	(Lac chrom-meter units)	
2-17-41	*	3.1		4.0	5.0	5.9		
2-25-41	4.9	2.6	3.38	3.6	4.6	5.6		
3-13-41	4.7	3.8		4.8	5.7	6.6		
3-29-41	4.9	3.1		4.1	5.0	5.9		
4-14-41	4.6	2.1		3.1	4.2	5.1		
4-20-41	4.6	2.1	3.76	3.1	4.2	5.2		
5- 7-41	4.2	5.1	9.85	6.0	6.8	7.4		
5-14-41	4.9	6.0		7.0	7.5	8.0	6.1	
5-27-41	4.5	5.5		6.4	7.4	8.4	6.0	
6- 2-41	4.6	5.7		6.7	7.6	8.5	6.5	
6-17-41	5.2	6.0		7.0	7.9	8.9	6.0	
7-28-41	5.0	5.8	12.15	6.7	7.7	8.7	5.8	

TABLE 1.—(*Continued*)

Raw Holstein Milk

Date	Normal milk			Normal milk with carotene added (Lac-chrom-meter units)			Standardized to 5% fat	Micrograms of carotene per quart to bring visual color of milk to that of Guernsey (both milks standardized to 5% fat)
	Fat content	Lac-chrom-meter units	Carotene content (mg./kg. fat)	300 µg.	600 µg.	900 µg.	(Lac-chrom-meter units)	
2–17–41		0.6		1.5	2.6	3.7		
2–25–41	4.7	0.0	1.28	0.7	1.6	2.5		
3–13–41	3.4	0.3		1.2	2.1	3.1		
3–29–41	3.3	0.0		0.6	1.5	2.4		
4–14–41	3.5	0.0		0.5	1.4	2.3		
4–20–41	3.4	0.0	1.18	0.6	1.5	2.4		
5– 7–41	3.2	0.0	2.98	0.9	1.8	2.7		
5–14–41	3.5	0.7		1.6	2.6	3.6	2.0	600
5–27–41	3.3	0.5		1.4	2.3	3.3	1.9	600
6– 2–41	3.2	0.6		1.5	2.4	3.4	1.5	600
6–17–41	3.5	0.5		1.4	2.4	3.4	1.9	600
7–28–41	3.5	0.5	3.55	1.4	2.4	3.4	1.8	600

TABLE 1.—(*Continued*)

Raw Jersey Milk

Date	Normal milk			Normal milk with carotene added (Lac-chrom-meter units)			Standardized to 5% fat	Micrograms of carotene per quart to bring visual color of milk to that of Guernsey (both milks standardized to 5% fat)
	Fat content	Lac-chrom-meter units	Carotene content (mg./kg. fat)	300 µg.	600 µg.	900 µg.	(Lac-chrom-meter units)	
2-17-41		3.0		4.0	4.9	5.8		
2-25-41	6.2	3.0	3.62	4.0	5.0	6.0		
3-13-41	4.3	2.0		3.0	4.0	5.1		
3-29-41	5.0	2.1		3.1	4.0	5.0		
4-14-41	5.0	1.6		2.6	3.6	4.5		
4-20-41	5.0	1.6	3.12	2.6	3.6	4.5		
5- 7-41	4.2	3.3	7.02	4.2	5.0	5.8		
5-14-41	5.8	5.5		6.4	7.4	8.2	5.0	180
5-27-41	5.7	5.5		6.4	7.4	8.4	4.9	
6- 2-41	5.7	5.2		6.1	7.1	7.0	4.8	180
6-17-41	5.5	5.0		6.1	7.0	8.0	4.8	180
7-28-41	5.5	4.8	8.65	5.9	6.9	7.8	4.6	180

TABLE 1.—(*Continued*)

Raw Mixed Herd Milk

Date	Normal milk			Normal milk with carotene added (Lac-chrom-meter units)			Standardized to 5% fat	Micrograms of carotene per quart to bring visual color of milk to that of Guernsey (both milks standardized to 5% fat)
	Fat content	Lac-chrom-meter units	Carotene content (mg./kg. fat)	300 µg.	600 µg.	900 µg.	(Lac-chrom-meter units)	
2–25–41	4.2	1.6		2.5	3.6	4.6		
5– 7–41	4.1	3.8	7.10	4.7	5.6	6.5		
5–14–41	4.6	3.4		4.5	5.4	6.4	3.6	600
5–27–41	4.2	3.2		4.2	5.3	6.4	4.0	450
6– 2–41	4.3	3.3		4.4	5.4	6.4	4.5	450
6–17–41	4.3	3.2		4.3	5.3	6.3	4.4	450
7–28–41	4.1	3.0	6.55	4.0	5.1	6.1	4.1	450

TABLE 1.—(*Continued*)

Pasteurized—Homogenized (2500 lbs.) Mixed Herd Milk

Date	Normal milk		Normal milk with carotene added (Lac chrom meter units)			Micrograms of carotene per quart to bring visual color of 4% milk to that of 5% Guernsey
	Fat content	Lac chrom meter units	300 µg.	600 µg.	900 µg.	
2-17-41	4.0	4.3	5.2	6.3	7.3	
2-25-41	4.0	4.4	5.3	6.3	7.3	
3-13-41	3.9	4.3	5.3	6.4	7.4	
3-29-41	4.0	4.3	5.4	6.5	7.6	
4-14-41	4.0	4.2	5.2	6.3	7.2	
4-20-41	4.0	4.3	5.2	6.2	7.2	
5- 7-41	4.0	5.7	6.8	7.8	8.8	
5-14-41	5.0	5.8	6.7	7.7	8.6	300
5-27-41	3.9	5.4	6.5	7.5	8.5	450
6- 2-41	3.9	5.6	6.5	7.5	8.5	300
6-17-41	4.0	5.8	6.9	8.0	9.0	300
7-28-41	4.0	5.6	6.6	7.6	8.6	300

1 and shows that the "Lac-chrom-meter" reading of the normal milk is different from that obtained when the milk was standardized to five per cent fat. No attempt was made to standardize the solids-not-fat.

Another important factor affecting the "Lac-chrom-meter" readings is the size of the fat globules in the milk, which is illustrated in table 1 where the readings obtained on the unhomogenized mixed herd milk are compared with the same milk after homogenization at 2500 pounds pressure. Homogenization at the above pressure causes an increase of more than 2.0 "Lac-chrom-meter" units over the corresponding unhomogenized milk. Although the homogenized milk has a richer color than the same milk unhomogenized, it is not in proportion to the increase in "Lac-chrom-meter" units.

Table 2 shows the increase in "Lac-chrom-meter" readings as the homogenization pressure is increased and the average size of the fat globules is decreased. These results indicate that as the fat globules are made smaller with a corresponding increase in numbers, the "Lac-chrom-meter" reading increases.

TABLE 2

Lac-chrom-meter reading and size of fat globules as influenced by various homogenization pressures. Mixed herd milk

Homogenization pressure	Lac-chrom-meter reading	Range of fat globule size (microns)	Size of majority of fat globules (microns)
Control—no pressure	3.1	0.5 – 10	5.0
500 lbs.	4.4	0.5 – 6	4.0
1000 lbs.	5.4	0.5 – 5	3.0
1500 lbs.	5.9	0.5 – 3	1.5
2000 lbs.	6.4	0.5 – 3	1.0
2500 lbs.	6.4	0.5 – 3	1.0
3000 lbs.	6.4	0.5 – 3	1.0
3500 lbs.	6.8	0.5 – 2	Less than 1.0
4000 lbs.	6.8	0.5 – 2	Less than 1.0

SUMMARY AND CONCLUSIONS

Guernsey milk showed the highest amount of color according to the "Lac-chrom-meter" reading and a visual inspection. Green pasture increased the color of the milk of all the breeds studied. "Provalac" (a carotene concentrate) added directly to the milk increased the "Lac-chrom-meter" reading approximately 1.0 unit for each 300 micrograms of carotene added per quart of milk. It would require about 450 micrograms of carotene per quart to be added to winter milk in order to increase the color to somewhere near the maximum obtained when cows are on pasture. The "Lac-chrom-meter" reading cannot be used as a direct measure of the amount of carotene in the milk. Homogenization increased the "Lac-chrom-meter" reading. The higher the pressure, the greater the reading. The visual color was not increased in proportion to the "Lac-chrom-meter" reading. In the case of homogenization the "Lac-chrom-meter" reading increased proportionally more than did the visual color, while when carotene was added the visual color increased in greater proportion than did the "Lac-chrom-meter" reading.

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THE EFFECT OF AMPHYL ON MOTILITY AND LONGEVITY OF BOVINE SPERMATOZOA[1]

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A common recommendation in the practice of artificial insemination of dairy cows is that all contact of semen with chemicals should be avoided. This precaution has applied especially to acids, alkalies, disinfectants, germicides, and such chemical compounds as may give off fumes that are corrosive in nature. A general recommendation of this kind undoubtedly is sound, but it is not based on complete experimental evidence of the effect of chemicals on spermatozoa.

In a series of experiments conducted to determine the value of certain disinfectants in maintaining dairy farm hygiene, an attempt was made to substitute Amphyl for heat in the sterilization of equipment and glassware used in the practice of artificial insemination. Amphyl (1, 2) is the registered trade name of a disinfectant consisting of a mixture of p-chloro-symm.-m-dimethyl hydroxybenzene, p-tert.-amyl hydroxybenzene, and neutral soap in a solution containing 20 per cent (by volume) of alcohol, 6.5 per cent of glycerol, and 34 per cent of water. The concentrated solution has a phenol coefficient of 10. Evidence of its non-toxic, non-injurious action to human and animal tissue and its efficacy as a disinfectant has been published (3, 4).

EXPERIMENTAL PROCEDURE

The semen from one Holstein bull was used in the experiments reported here. In all cases the sperm were 95 per cent motile immediately following ejaculation. In determining the percentage of motility, the semen was transferred to a glass microscope slide by means of a sterile standard wire loop. The glass slide was warmed to about body temperature and the semen was covered with a thin glass slip before examination was made under the microscope. Both the high-dry and oil-immersion objectives of the microscope were used.

The semen, unless otherwise stated, was diluted with a standard diluter solution consisting of equal parts, by volume, of egg yolk and phosphate buffer. The phosphate buffer solution was made by dissolving 0.1 gram of anhydrous KH_2PO_4 and 0.39 gram of anhydrous Na_2HPO_4 in 50 ml. of distilled water. The buffer solution was sterilized in the autoclave.

All semen samples were held at 3° C. while in storage.

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The concentrated Amphyl was diluted with water to the desired concentration.

The measures of the effect of the various treatments on sperm were the percentage of motility at any given time and the length of time required for cessation of all motility.

EXPERIMENTAL RESULTS

Seven experimental trials served to demonstrate that the residues left on artificial insemination equipment and glassware after sterilization with Amphyl had no harmful effect on the motility or longevity of the sperm of semen subsequently brought into contact with this equipment. A severer test of the effect of the Amphyl on semen, therefore, seemed indicated.

The addition of one ml. of a one per cent solution of Amphyl to one ml. of undiluted semen caused all sperm motility to cease within 30 seconds. When one ml. of semen was diluted with one ml. of the phosphate buffer solution which contained one per cent of Amphyl, all motility ceased within one hour. When a small volume of the concentrated Amphyl, however, was added to semen that had been diluted with standard diluter, motility persisted for several days. This unexpected behavior led to the organization of five experimental trials in order to observe, more carefully, the action of the disinfectant on semen.

In the first trial, a sample of semen was diluted 1 : 3 with the standard diluter and was divided into 2 parts, one part to be the control sample and one part the experimental sample. One volume of one per cent Amphyl was added to 5 volumes of the experimental sample, which gave a final concentration of 0.167 per cent of the disinfectant in the diluted semen. Bacterial counts in the treated and untreated semen were made on the first, third, and seventh days of storage. The percentages of motile sperm in both samples were determined daily with the exception of the second and fourth days. The results are presented in table 1.

The rate of decrease in percentage of sperm motility was identical for both the control and the Amphyl-treated semen during the first 5 days of storage. Thereafter, however, the rate of decrease was considerably less in the treated semen than in the untreated semen. All motility had ceased in the control sample by the seventh day, whereas, a small percentage of sperm were still motile in the treated semen, on the tenth day. The bacteriological data indicate that the disinfectant had little influence on the bacteria under the conditions prevailing in this experiment.

In trial 2, a 1 : 1 dilution of the semen was made with the standard diluter. The diluted semen was divided into 3 equal portions of 4 ml. each and put into glass tubes. Tube 1 served as the control. One ml. of one per cent Amphyl was added to tube 2; 2 ml. were added to tube 3. The concentration of Amphyl in tubes 2 and 3 was, respectively, 0.2 and 0.33 per cent.

The results of the motility and bacteriological examinations and of the pH determinations are shown in table 2.

The rate of decrease in motility was about the same for the control and Amphyl-treated samples of semen for the first 4 days. Thereafter the Amphyl-treated semen showed a slower decrease in motility than the control semen. All motility had ceased in the control semen after the seventh day, in the semen containing 0.2 per cent Amphyl after the eleventh day, and in the semen containing 0.33 per cent Amphyl after the fourteenth day. It is interesting to note the length of time motility persisted in a small number of sperm in the semen containing 0.33 per cent of Amphyl. The difference in the numbers of bacteria in the three samples did not seem significant.

TABLE 1

Effect of Amphyl on the percentage of motile sperm, the persistence of sperm motility, and the numbers of bacteria in semen—trial 1

Days	Motility		Bacteria per ml. of undiluted semen	
	Control	Treated	Control	Treated
	%	%	*thousands*	*thousands*
1	80	80	1,840	960
3	75	75	392	230
5	50	50	...	...
6	50	50	...	...
7	0	40	320	415
8	0	20		...
9	0	20	...	...
10	0	5	...	...
11	0	0	...	...

The pH values of the three samples were identical on the first day and all showed an equal, but slight, increase in acidity by the eighth day.

Trial 3 was similar to trial 2 except that the diluted semen was divided into 4 parts, the concentration of Amphyl in tube 4 being 0.5 per cent. The results on motility, shown in table 3, are similar to those obtained in the two preceding trials. There is some indication that a concentration 0.5 per cent Amphyl is too high for optimum effect. This is substantiated by the data in table 4, obtained from trial 4 in which the diluted semen contained 0.67 per cent Amphyl. The rate of decrease in motility was considerably greater than in the cases where a lesser concentration of Amphyl was used. These results indicate that the optimum concentration of Amphyl lies between 0.33 and 0.5 per cent.

The possibility that other disinfectants might have an effect similar to Amphyl on sperm in standard diluter was investigated in trial 5, in which Lysol and Zephiran were used in comparison with Amphyl. Lysol is a mixture of cresylic acid, neutral soap and glycerol. Zephiran is a mixture of high molecular weight alkyl-dimethyl-benzyl-ammonium chlorides. One volume of semen was diluted with 2 volumes of standard diluter and the mixture was divided into 4 parts, each part consisting of 2 ml. of the diluted semen. Tube 1 served as the control, tube 2 contained one ml. of one per cent Amphyl, tube 3 contained one ml. of one per cent Lysol, and tube 4 contained one ml. of one per cent Zephiran The results are presented in table 5.

The results presented in table 5 show that Lysol is highly toxic to sperm even though some motility persisted for three days. Zephiran did not exhibit much effect on sperm, although the rate of decrease in percentage of motility was slightly greater than in the control semen. Motility persisted for four days longer in the Amphyl-treated semen than in the control semen. The sperm in this sample of semen were unusually virile, as indicated by the persistence of motility in the control sample for ten days. Generally, motility persists for only five or six days.

TABLE 2

Effect of Amphyl in different concentrations on the percentage of motile sperm, the persistence of sperm motility, the numbers of bacteria, and the pH of semen—trial 2

Days	Motility			Bacteria per ml. of undiluted semen			pH		
	Control	Treated*		Control	Treated*		Control	Treated*	
	1	2	3	1	2	3	1	2	3
	%	%	%	*thousands*					
1	95	95	95	356	339	275	6.4	6.4	6 4
2	90	85	85						
3	80	80	75						
4	50	50	50						
5	20	40	40						
6	5	30	30						
7	5	20	20						
8	0	20	20				6.2	6.2	6.2
9	0	10	10						
10	0	5	5	299	381	284			
11	0	5	5				6.2	6.2	6.2
12	0	0	5		236	279			
13	0	0	5						
14	0	0	5						
15	0	0	0						

* Treated semen: tube 2 contained 0.2 per cent Amphyl; tube 3 contained 0.33 per cent.

An experiment was conducted to observe the effect of Amphyl on an organism other than bovine sperm. A pure culture of a hemolytic staphylococcus, which contained 500,000,000 organisms per ml., was treated with Amphyl in the presence of phosphate buffer alone, in the presence of egg yolk alone, and in the presence of the standard diluter. Ten ml. of each solution were sterilized, inoculated with 0.1 ml. of the staphylococcus culture, and allowed to stand 30 minutes before being plated out in beef extract-blood (bovine) agar. The plates were incubated at 37° C. for 48 hours.

The results, presented in table 6, show that Amphyl destroyed all organisms in the presence of either the phosphate buffer alone or the egg yolk alone, whereas, in the presence of the standard diluter, many of the organisms continued to grow. The number, however, was only about one per cent of the total which grew in the standard diluter medium in the absence of Amphyl. Tests showed, further, that organisms were still alive in this medium in the presence of one per cent Amphyl after seven days. These results agree well with the findings on bovine sperm.

TABLE 3

Effect of Amphyl in different concentrations on the percentage of motile sperm, the persistence of sperm motility, and the numbers of bacteria in semen—trial 3

Days	Motility				Bacteria per ml. of undiluted semen			
	Control	Treated*			Control	Treated*		
	1	2	3	4	1	2	3	4
	%	%	%	%	*thousands*			
5 hours	95	95	90	90				
1	90	95	90	90	375	320	437	376
2	80	80	80	70				
3	80	70	70	70				
4	50	60	50	50				
5	30	60	50	50				
6	0	45	40	20				
7	0	15	40	30	331	372	440	319
8	0	0	20	20				
9	0	0	35	20				
10	0	0	30	15				
11	0	0	30	15				
12	0	0	30	15				
13	0	0	30	10				
14	0	0	5	5				
15	0	0	0	0				

* Treated semen: tube 2 contained 0.2 per cent Amphyl; tube 3 contained 0.33 per cent; tube 4 contained 0.5 per cent.

TABLE 4

Effect of Amphyl in high concentration (0.67%) on the percentage of motile sperm, the persistence of sperm motility, and the numbers of bacteria in semen—trial 4

Days	Motility	Bacteria per ml. of undiluted semen
	%	*thousands*
1	85	893
2	60	
3	40	
4	50	
5	30	
6	5	
7	0	
8	0	594

TABLE 5

Effect of equal concentrations of Amphyl, Lysol, and Zephiran on the percentage of motile sperm and the persistence of sperm motility in semen diluted with standard diluter—trial 5

Days	Motility			
	Control semen	Treated semen		
		Amphyl	Lysol	Zephiran
	%	%	%	%
1	95	90	50	90
2	85	75	15	75
3	60	50	5	50
4	50	50	0	50
5	40	30	0	20
6	40	25	0	15
7	20	30	0	15
8	15	15	0	15
9	5	15	0	10
10	1	15	0	0
11	0	15	0	0
12	0	15	0	0
13	0	15	0	0
14	0	15	0	0
15	0	15	0	0

TABLE 6

Bactericidal effect of Amphyl on a hemolytic staphylococcus suspended in various media

Solutions	Bacteria per ml *thousands*
10 ml phosphate buffer	0
10 ml. phosphate buffer + 0.1 ml. culture	73,000
10 ml phosphate buffer + 0.1 ml. culture + 1 ml. Amphyl	0
5 ml. egg yolk + 5 ml. water	0
5 ml egg yolk + 5 ml. water + 0 1 ml culture	173,000
5 ml. egg yolk + 5 ml water + 0 1 ml culture + 1 ml Amphyl	0
10 ml. standard diluter	0
10 ml. standard diluter + 0.1 ml culture	247,000
10 ml. standard diluter + 0 1 ml culture + 1 ml. Amphyl	2,480

DISCUSSION

No completely satisfactory explanation of the behavior of bovine sperm or of the hemolytic staphylococcus in the presence of Amphyl can be offered. It seems possible that the toxicity of the disinfectant toward sperm and bacterial organisms is greatly reduced or completely nullified by the presence of the ingredients in the standard diluter. This conceivably could take place through some type of chemical combination, perhaps between Amphyl and egg yolk in the presence of phosphates. The increased longevity and motility of the sperm, however, cannot be explained on this basis.

SUMMARY

The motility of bovine sperm in undiluted semen ceased within 30 seconds after the addition of 0.5 per cent Amphyl, a disinfectant containing synthetic organic compounds.

Indications are that sperm, suspended in an egg yolk-phosphate buffer dilution medium containing from 0.2 to 0.5 per cent of Amphyl, maintained motility longer than that of sperm suspended in the same medium but containing no Amphyl.

Sperm suspended in phosphate buffer solution containing one per cent Amphyl soon lost all motility.

Sperm suspended in egg yolk-phosphate buffer solution containing Lysol lost all motility within three days, whereas, those suspended in the same medium but containing Zephiran remained motile for a period equal to that of the control sample.

Hemolytic staphylococci were completely destroyed in phosphate buffer and in egg yolk solution containing one per cent Amphyl; many of the staphylococci continued to grow after seven days when suspended in egg yolk-phosphate buffer solution containing one per cent Amphyl.

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THE VITAMIN A VALUE OF ROQUEFORT TYPE CHEESE*

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INTRODUCTION

Several factors have been reported to have an effect on the vitamin A value of cheese. Hathaway and Davis (6) in a study of twenty different kinds of cheese found considerable variation in vitamin A content of any given kind of cheese. They reported that the vitamin A value was influenced by the vitamin A potency of the milk used, the amount of butterfat in the milk and the methods of processing, packaging and storage. Carotenoids have been shown to be present in microorganisms and the ability of certain microorganisms to synthesize carotene has been demonstrated (1, 9). It was considered possible that the organisms used in the manufacture of Roquefort cheese also might have the capacity to produce carotene or vitamin A. Therefore, the following investigation was planned to determine the vitamin A value of cheese ripened with Roquefort mold, similar cheese without mold growth and the unripened frozen curd.

EXPERIMENTAL

Preparation of cheese: The cheeses were all made on September 10, 1940, from the same lot of raw milk in the same vat. The method of manufacture used was a modification of two well known processes (5, 7) for the making of blue cheese. The modifications were to obtain the cheese in cylinders of 3⅛″ with the desired moisture content. Just before the curd was put in the cylinders it was inoculated with a mold powder prepared from a culture of *Penicillium roqueforti*, Culture 33D (2, 3). The next day the cylinders of curd were cut into pieces 1½″ high and brine salted for 2 hours in a saturated brine solution. At this stage the curd was divided into three groups:

(a) Frozen Roquefort type curd. The salted curd was vacuum packed in #2 short one-half pound flat Columbia River sized re-enamel cans. This curd was prevented from ripening into cheese by holding it at 0° F. or lower until used.

(b) Roquefort type cheese, ripened without mold growth. This lot was vacuum canned exactly the same as lot (a), thus inhibiting mold growth, but was allowed to ripen in the vacuum sealed cans at the same temperature as lot (c).

(c) Roquefort type cheese, ripened with mold growth. This lot was

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placed in the cans with lids loosely placed on. Both lots (b) and (c) were then held in a gas controlled chamber (4) at a temperature of 50° F. until October 29, 1940, when lot (c) had developed a good growth of *P. roqueforti.* The cans of lot (c) were then vacuum sealed. Both lots (b) and (c) were then placed in cool storage (30° F. to 40° F.) until used.

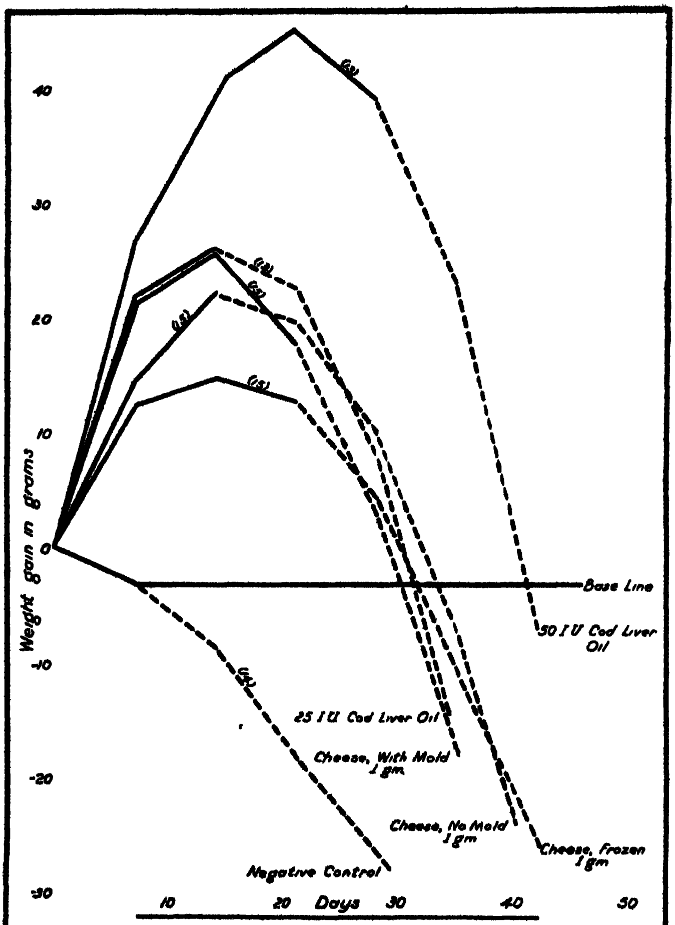

Fig 1 - Average growth curves of animals receiving single feedings of cheese or cod liver oil as a source of vitamin A.
Note: Figures on the curves indicate number of animals in each group and broken lines indicate death of one or more animals.

Method of assay: Vitamin A assays were carried out by the single-feeding method of Sherman and Todhunter (8). Eighty-five albino rats, 40 to 50 grams in weight, and 21 to 28 days of age, and with low initial reserves of vitamin A were prepared for the assay according to the directions of the U. S. Pharmacopoeia XI. The basal diet consisted of: casein (heat-treated)

18 per cent; cornstarch 65 per cent; yeast 8 per cent; salt mixture (Osborne and Mendel) 4 per cent; Wesson oil 5 per cent. Viosterol, three drops per week, was given orally throughout the experiment. The rats were depleted of their reserves of vitamin A in 25 to 36 days and were allotted to one of six groups which were equalized for sex, litter, weight and length of time of depletion. The groups were as follows: (a) Frozen Roquefort type curd, one gram, (b) Roquefort type cheese, ripened without mold growth, one gram, (c) Roquefort type cheese, ripened with mold growth, one gram, (d) basal diet only, (e) reference cod liver oil, 25 I.U., (f) reference cod liver oil, 50 I.U. Single feedings of these supplements were given and the animals were allowed basal diet *ad libitum* until death. Growth curves were drawn for all animals and the area under the curve, using the base line as described by Sherman and Todhunter (8) was measured with a planimeter.

RESULTS AND DISCUSSION

The data are summarized in table 1 and figure 1. In order to determine whether the areas under the curves were significantly different, the data were treated statistically by the analysis of variance method. The areas for the curves of the three samples of cheese were not significantly different from each other nor from that obtained when 25 I.U. of vitamin A were fed as reference cod liver oil. Therefore, it is concluded that the vitamin A value of each of these samples of cheese was approximately 25 I.U. per gram, and that the Roquefort organism, Culture 33D, was without influence on the vitamin A value of the cheese under these conditions. Hathaway and Davis (6) reported values of 40 I.U. of vitamin A per gram of Roquefort cheese.

TABLE 1

Vitamin A value of Roquefort type cheese

Supplement	Moisture per cent	Number of animals		Area under curve	Vitamin A value (I.U. per gm.)
		M	F		
(a) Frozen Roquefort type curd, 1 gm.	45.52	8	7	3.91	25
(b) Roquefort type cheese ripened without mold growth, 1 gm.	44.94	8	7	5.48	25
(c) Roquefort type cheese curd ripened with mold growth, 1 gm.	41.67	8	7	5.51	25
(d) Basal diet			.	.	.
(e) Reference cod liver oil, 25 I.U.		5	8	6.20	1700
(f) Reference cod liver oil, 50 I.U.		6	7	13.37	1700

The area under the curve for the group receiving 50 I.U. of vitamin A as reference oil was almost exactly twice that of the group receiving 25 I.U. of vitamin A and the difference in area was found to be statistically significant.

This offers confirmatory evidence that the area under the curve is proportional to the amount of vitamin A fed and that the method of single feeding is reliable for vitamin A measurements.

SUMMARY

The vitamin A value of Roquefort type cheese with and without mold development was determined by bioassay using the method of single feeding. The vitamin A content was the same for all samples of cheese, approximately 25 I.U. per gram. No evidence was obtained that mold growth, by Culture 33D, in Roquefort type cheese increased the vitamin A value.

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OXIDIZED FLAVOR IN MILK. XI. ASCORBIC ACID, GLUTATHIONE, AND HYDROGEN PEROXIDE AS MECHANISMS FOR THE PRODUCTION OF OXIDIZED FLAVOR*

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Hand and Sharp (11) recently proposed that changes in the flavor of milk might be produced by certain pigments, vitamins, and enzymes. The riboflavin system was mentioned as was also the oxidation of ascorbic acid with cucumber "oxidase." They found that other catalysts of ascorbic acid oxidation produce hydrogen peroxide. Thurston, Brown, and Dustman (24) reported that oxidized flavor in milk was caused by the oxidative breakdown of lecithin in milk. The term lecithin was used as inclusive for the phospholipids. Lecithin and cephalin have been shown to be easily oxidized (5). From this it would seem that the mechanism for the development of oxidized flavor in milk might be the oxidation of ascorbic acid with the production of hydrogen peroxide which then oxidizes the phospholipids, liberating the compounds which give the oxidized flavor.

In studying the oxidation of phospholipids in relation to cancer, Deutsch, Kline, and Rusch (9) found by manometric technique that the reaction was catalyzed by the presence of ascorbic acid at a pH of 4.0. In a further study Rusch and Kline (21) found that the oxidation of various phospholipid preparations was catalyzed by ascorbic acid, glutathione, and cysteine at pH 3.5 but that these substances were inactive at pH 7.4. Thiamine, riboflavin, pyridoxine, and methylene blue catalyzed the oxidation at pH 7.4 but not at pH 3.5, They found that certain carcinogenic hydrocarbons and hydroquinone, catechol, carotene, and cholesterol, as well as other compounds, inhibited the oxidation. Most of the compounds catalyzing the reaction and some of the inhibitors are found in milk.

Barron, Barron and Klemperer (1) studied the oxidation of ascorbic acid catalyzed by copper. Using the manometric technique they found that the amount of ascorbic acid oxidized was proportional to the copper content of milk. Orange juice, tomato juice, and grapefruit juice also showed oxidation of ascorbic acid with copper at higher acidities.

Stotz, Harrer, and King (22) made a startling discovery when they attributed the catalytic activity of "ascorbic acid oxidase" from cauliflower and squash juices to the copper present in combination with protein. A

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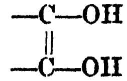
mixture of copper and albumin was found to have the same characteristics as the "enzymes" in relation to oxidation of ascorbic acid, optimum pH, and inactivation by heat. In a further study McCarthy, Green, and King (16) compared the copper protein complexes and cucumber "oxidase" with the similar system potato oxidase on catechol. The potato system was found to be different from the cucumber system, but the cucumber "oxidase" was similar to the copper protein complex. Meiklejohn and Stewart (17) compared the cucumber "oxidase" with a copper protein complex and found that the cucumber oxidase was more active. They did, however, find that complete removal of copper inactivated the cucumber enzyme.

Hand and Griesen (10) made a study of different catalysts for the oxidation of ascorbic acid. Using Barcroft manometers to measure the oxygen consumption they found that cucumber "oxidase" caused the absorption of one atom of oxygen per molecule of ascorbic acid; copper at various pH values caused absorption of between one and two atoms of oxygen; riboflavin and light caused absorption of two atoms of oxygen at pH 4 to 5 They explained that the reason for combination of one molecule of ascorbic acid with more than one atom of oxygen was the intermediate formation of hydrogen peroxide. They stated: "If the the hydrogen peroxide oxidizes ascorbic acid or is decomposed by catalase the number of oxygen atoms equals one; but if hydrogen peroxide accumulates in the solution or oxidizes dehydroascorbic acid the number of oxygen atoms equals two."

In a review of the oxidase systems of higher plants, Boswell and Whiting (4) found that the ascorbic acid oxidase, dihydroxy maleic acid oxidase, and catechol oxidase all belonged to the same system The reason for this was the same grouping in the substrates, the  $\begin{array}{l}\text{—C—OH}\\ \;\;\|\\ \text{—C—OH}\end{array}$ group. With all three compounds hydrogen peroxide was formed when the right catalysts were used for the oxidation. Barron, De Meio, and Klemperer (2) explained the formation of hydrogen peroxide by the fact that ascorbic acid was oxidized by the cupric ion forming dehydroascorbic acid and cuprous ion. The cuprous ion was then oxidized by oxygen of the air to the cupric ion producing hydrogen peroxide. Dekker and Dickinson (8) gave a similar explanation for the production of hydrogen peroxide by copper catalytic oxidation of the ascorbic acid.

Robertson, Ropes and Bauer (19) studied a system somewhat similar to milk in the degradation of mucins and polysaccharides by ascorbic acid and hydrogen peroxide. That hydrogen peroxide was the cause of the mucin degradation was proved by getting no reaction when catalase was added to the system compared to a degradation of mucin when it was treated with ascorbic acid in copper sulphate solution.

Corbett and Tracy (6) reported an acceleration of oxidized flavor in some cases and protection in other cases with ascorbic acid in milk. Dahle and Palmer (7) as well as a number of others have found ascorbic acid to protect milk from oxidized flavor.

Hopkins (13) studied the influence of glutathione on fats and proteins. At pH 3 to 4 the fatty acids, linoleic and linolenic, were oxidized by glutathione and oxygen. At pH 7.4 to 7.6 he found that half the oxygen uptake was used for the oxidation of the sulfhydryl group of glutathione and half for the oxidation of lecithin. Tait and King (23) in studying the oxidation of lecithin in the presence of glutathione found that the oxygen uptake of lecithin was about four times that which would be expected from a consideration of its iodine number in relation to the iodine number of its constituent fatty acids and their oxygen uptake. Hopkins and Morgan (14) studied the relationship of glutathione with ascorbic acid. They found that as long as glutathione was present in the unoxidized form, ascorbic acid was completely protected from oxidation, but after the glutathione was oxidized the ascorbic acid oxidized normally. Rosenthal and Voegtlin (20) found that copper ions oxidized reduced glutathione only to the oxidized form but they oxidized cysteine, which has a similar sulfhydryl group, from the reduced form to the break down constituents of ammonia, carbon dioxide and sulphuric acid.

Many theories can be postulated on the mechanism of oxidized flavor in milk, but some method of testing the theories must be found and in applying them to milk it is necessary to have sufficient sample so that the flavor may be determined by taste. In the papers reviewed most of the investigators have used manometers to measure the oxygen absorbed in order to determine the rate of oxidation. The use of milk is complicated by the number of interfering substances. Palmer and Wiese (18) washed cream from five to eight times to remove the milk plasma solids. This left only the fat globules composed of butter fat and fat globule membrane, the latter being a mixture of protein and phospholipids. If it is the phospholipids that give the oxidized flavor, they should be easily oxidized in washed cream and this could be used to test the theories. Accordingly, the following experiment was planned and conducted.

EXPERIMENTAL

For use in this experiment, cows whose milks were susceptible to oxidized flavor were selected and the milk from these animals was used throughout the experiment.

Samples of milk were collected from these cows each morning and placed in a ten gallon aluminum can. The milk was then pasteurized by immersion of the can in a vat fitted with steam coils and a cold water inlet. The temperature of the milk was raised to 144° F. ± 1° F. and held for 30 minutes. Following pasteurization a quart sample of the milk was removed and used as a control. The remainder of the milk was then separated and the cream was washed by dilution with a volume of hot water equal to the volume of the skim milk removed. This was repeated five times so that practically all of the milk plasma solids were removed. In order to control the pH a buffer (6.8) was used in certain cases. This buffer was heated to 140–150° F. and added to the washed cream and the cream was separated a sixth time. One examination of the washed cream revealed only 0.25 per cent protein indicating practically complete protein removal. The washed cream thus obtained was then ready for experimental treatment.

The control sample of milk was divided into four half-pints. One of these was used as a control with no added copper while the others were contaminated with 0.5, 1.0, and 1.5 p.p.m. of copper, respectively, added from a copper sulphate solution. The cream was divided into two lots. Each lot was then divided and contaminated with copper in the same way as the milk. In addition to the added copper, in the second lot 50 mg. of ascorbic acid was added to each half-pint sample, which is equivalent to 210 mg. per liter. All samples were then stored at 40° F. ± 5° F. for three days, after which they were scored by at least three judges familiar with oxidized flavor. Since the oxidized flavor developed in the washed cream was so much stronger than that developed in the control milk, no attempt was made to classify its intensity. The results of this experiment are shown in table 1. These results show quite clearly that copper added to washed cream will not catalyze the development of oxidized flavor. In none of the five trials was there any trace of oxidized flavor developed. In the trials in which 210 mg. per liter of ascorbic acid were added to the washed cream prior to contamination with copper, a strong oxidized flavor developed. In most cases where the ascorbic acid was added to the control sample of washed cream containing no added copper the oxidized flavor likewise developed.

Since ascorbic acid has been reported to protect milk against the development of oxidized flavor it seemed desirable to study the effect of various concentrations of ascorbic acid on the susceptibility of washed cream. Accordingly, washed cream was fortified with various cncentrations of ascorbic acid and contaminated with copper as in the preceding experiment. The results are shown in table 2. An examination of these results shows that when washed cream was fortified with 630 mg. per liter of ascorbic acid no oxidized flavor developed. The addition of buffer to the washed cream to control the pH of the reaction did not change the results. At concentrations of ascorbic acid of 21 mg. per liter the development of oxidized flavor was erratic. However, concentrations of from 40 to 100 milligrams per liter gave the strongest development of the flavor.

In the previous experiments, it was observed that the oxidized flavor developed by addition of ascorbic acid to washed cream was many times as strong as that produced in susceptible milk. In many instances it was necessary to use normal milk and dilute the highly flavored washed cream so

TABLE 1

Relationship of ascorbic acid to oxidized flavor in washed cream

Date	Control milk				Washed cream								
					No added ascorbic acid				210 mg./l. ascorbic acid				Buffered or unbuffered
	p.p.m. copper				p.p.m. copper				p.p.m. copper				
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	
2/2/42	–*	2*	3*	3*					ox†	ox	ox	ox	Unbuffered
2/4/42	–	2	3	3					ox	ox	ox	ox	"
2/13/42	–	1	1	1	–	–	–	–	ox	ox	ox	ox	"
2/14/42	–	1	1	1	–	–	–	–					Buffered
2/16/42	–	1	1	1	–	–	–	–	ox	ox	ox	ox	Unbuffered
2/17/42	–	1	1	2	–	–	–	–	ox	ox	ox	ox	Buffered
2/19/42	–	1	1	1					ox	ox	ox	ox	"
2/19/42	–	1	1	1					acid	ox	ox	ox	Unbuffered
2/24/42	–	1	2	2	–	–	–	–					Unbuffered
2/25/42	–	1	1	1					ox	ox	ox	ox	Unbuffered

* Intensity of oxidized flavor: – no oxidized flavor; ? doubtful; 1 slight oxidized flavor; 2 moderate oxidized flavor; 3 pronounced oxidized flavor; and 4 very pronounced oxidized flavor.

† Oxidized flavor.

TABLE 2

Relationship of concentration of ascorbic acid to oxidized flavor in washed cream

Date	Control milk				Washed cream																				Buffered or unbuffered
					630 mg./l. ascorbic acid				210 mg./l. ascorbic acid				105 mg./l. ascorbic acid				42 mg./l. ascorbic acid				21 mg./l. ascorbic acid				
	p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	
2/18/42	–*	2*	2*	3*					ox*	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	Buffered
2/23/42	–	1	2	2					ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	"
2/25/42	–	1	1	1					ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox					Unbuffered
2/27/42	–	1	1	2									ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	"
3/13/42	–	1	2	2					–	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox					"
3/16/42	–	1	1	1	–	–	–	–	–	ox	ox	ox													"
					–	–	–	–	ox	ox	ox	ox													Buffered
3/17/42	–	2	2	3	–	–	–	–	–	ox	ox	ox													Unbuffered
					–	–	–	–	ox	ox	ox	ox													Buffered
3/19/42	–	1	2	2	–	–	–	–	–	ox	ox	ox													Buffered
3/24/42	–	1	1	1	–	–	–	–	–	ox	ox	ox	ox	ox	ox	ox									Unbuffered
3/25/42	–	1	1	1					ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	–	–	–	–	"
3/26/42	–	1	1	1					ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox					"
3/27/42	–	1	1	1					ox	ox	ox	ox	ox	ox	ox	ox	–	?	?	–					"

* See table 1, footnote.

TABLE 3

The relationship of ascorbic acid to oxidized flavor in washed cream from non-susceptible milk

Date	Control milk				Washed cream																Buffered or unbuffered
					210 mg./l. ascorbic acid				105 mg./l. ascorbic acid				42 mg./l. ascorbic acid				21 mg./l. ascorbic acid				
	p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	
/17/42	–*	–	–	–	?	ox*	ox	ox	ox	ox	ox	ox	?	ox	ox	ox					Unbuffered
/18/42	–	–	–	–	–	ox	ox	ox	ox	ox	ox	ox	–	ox	ox	ox	–	–	–	–	“
/20/42	–	–	–	–	–	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	–	–	?	?	“
/21/42	–	–	–	–	–	–	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	–	–	–	–	“
/22/42	–	–	–	–	–	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	–	–	–	?	“

* See table 1, footnote.

as to obtain a typical oxidized flavor. Since the flavor developed in washed cream was so much stronger than that developed in normal milk it seemed desirable to study the effect of ascorbic acid on non-susceptible milk. Several attempts were made to select cows which gave non-susceptible milk, but during the dry feeding period when sufficient quantity of milk was selected there was usually a suggestion of oxidized flavor after the three-day storage period. Accordingly, several cows were selected and placed on pasture during the day. After about one week of early spring pasture the morning milk from these cows was tested and found to be non-susceptible. This milk was then used in obtaining the data in table 3. The results obtained in this study show that an oxidized flavor can be developed in washed cream from normally non-susceptible milk. An examination of these data reveals quite clearly that the material rendering milk non-susceptible to oxidized flavor, when contaminated with copper, is carried in the water phase of the milk. These results are in agreement with the observations of Krukovsky (15).

Since ascorbic acid has been shown to catalyze the oxidation of phospholipids and produce an oxidized flavor in washed cream, it seemed advisable to determine if several of the other materials suggested by Rusch and Kline (21) as catalysts for this reaction, might likewise catalyze the development of oxidized flavor. Accordingly, several of these materials were used in various concentrations. The results of this study are shown in table 4. An examination of these data reveals that thiamine, pyridoxine, riboflavin, and cysteine, do not catalyze the development of oxidized flavor in washed cream even in the presence of copper. The data obtained with riboflavin were rather limited but when increased concentrations were used they caused the development of a bitter flavor which would have masked any oxidixed flavor present. The work of Hand and Griesen (10) suggests a role for riboflavin through the oxidation of the ascorbic acid. However, because of the bitter flavor developed this could not be checked by taste. Cysteine was tried at several different concentrations but at higher concentrations its flavor was such as to mask any oxidized flavor developed.

Glutathione in the presence of copper caused the development of an oxidized flavor. It was noted that as the concentration of the copper increased, the intensity of the oxidized flavor developed also increased.

DISCUSSION AND RESULTS

The results obtained in this experiment indicate that either ascorbic acid or glutathione, or both, are probably involved in the mechanism whereby an oxidized flavor is developed in milk. Corbett and Tracy (6) reported that in copper contaminated milk the ascorbic acid first retarded the development of the oxidized flavor and then after a certain point was reached, the development of the oxidized flavor was accelerated. Results obtained in the present study show that high concentrations of ascorbic

TABLE 4

The relationship of certain compounds to oxidized flavor in washed cream

Date	Control milk				Washed cream																				Buffered or unbuffered
					210 mg./l. thiamine				105 mg./l. pyridoxine				4 mg./l. riboflavin				10 mg./l. cysteine				105 mg./l. glutathione				
	p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	
2/6/42	–	2*	3*	3*	–	–	–	–																	Unbuffered
2/12/42	–	2	2	3	–	–	–	–																	Buffered
3/14/42	–	1	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–					Unbuffered
2/16/42	–	1	2	3					–	–	–	–													“
3/16/42	–	1	1	1					–	–	–	–													“
2/23/42	–	1	2	2													–	–	–	–					Buffered
3/12/42	–	1	1	2																	–	ox	ox	ox	Unbuffored
3/17/42	–	2	2	3																	–	–	ox	ox	“
3/24/42	–	1	1	1																	–	ox	ox	ox	“

* See table 1, footnote.

TABLE 5

The effect of hydrogen peroxide on the development of oxidized flavor in washed cream

Date	Control milk				Washed cream											
					Containing 0.01% hydrogen peroxide				Containing 0.05% hydrogen peroxide				Containing 0.1% hydrogen peroxide			
	p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper			
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5
2/21/42	–	1*	2	2	–	?*	?	?					–	ox	ox	ox
3/2/42	–	1	1	2	–	–	–	–	–	–	–	–	–	–	ox	ox
3/3/42	–	1	1	2	–	–	–	ox	–	ox	ox	ox	–	ox	ox	ox
3/6/42	–	?	1	2	–	ox	ox	ox	–	ox	ox	ox	–	ox	ox	ox
3/9/42	–	1	2	2	–	?	ox	ox	–	ox	ox	ox	–	ox	ox	ox
3/10/42	–	1	1	1	–	ox	ox	ox	–	ox	ox	ox	–	ox	ox	ox
						Increasing → Intensity				Increasing → Intensity				Increasing → Intensity		

* See table 1, footnote.

acid protect cream against oxidized flavor while lower concentrations cause the flavor to develop. The use of a buffer to control the pH did not affect these results.

Both glutathione and ascorbic acid catalyze the development of oxidized flavor. Any theory as to the reaction whereby this oxidation is brought about should be applicable to both substances. A review of the literature published on the oxidation of ascorbic acid reveals that the oxidation of these two substances may have certain similarities. The oxidation of ascorbic acid by copper to dehydroascorbic acid has been reported to liberate hydrogen peroxide (2, 8, 10, 19).[3] Likewise, it has been postulated (25) that hydrogen peroxide is liberated when glutathione is oxidized with copper. The references just cited indicate that cupric copper is reduced by either ascorbic acid or glutathione to the cuprous form. The cuprous form is then oxidixed by molecular oxygen to form cupric copper and hydrogen peroxide.

Since hydrogen peroxide is a strong oxidizing substance it may be possible that it reacts with the phospholipids to oxidize them and thus bring about the development of an oxidized flavor. This particularly seems true since it was impossible to develop an oxidized flavor in washed cream with copper alone. Such a theory suggested the direct addition of hydrogen peroxide to washed cream. This was tried with the results shown in table 5. An examination of these data shows that hydrogen peroxide in proper concentration with copper as a catalyst does produce an oxidized flavor. As the rate of copper contamination increased the intensity of the oxidized flavor developed likewise increased. The role of copper in this oxidation with hydrogen peroxide is not known.

Although the development of oxidized flavor by means of hydrogen peroxide does not give positive proof that hydrogen peroxide is liberated during the reaction in which ascorbic acid or glutathione is oxidized in the development of the flavor, it does give indirect evidence that such a mechanism may exist and may be responsible when an oxidized flavor is developed.

CONCLUSIONS

1. Washed cream from susceptible milk does not develop an oxidized flavor when contaminated with copper and stored for three days.

2. Washed cream fortified with ascorbic acid (40 to 200 mg. per liter) and contaminated with copper develops a strong oxidized flavor.

3. Washed cream containing added glutathione develops an oxidized flavor when contaminated with copper.

4. Washed cream from non-susceptible milk develops an oxidized flavor when contaminated with copper and fortified with ascorbic acid.

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[3] Since this manuscript was prepared, Steinman and Dawson have submitted additional proof for the production of hydrogen peroxide when ascorbic acid is oxidized by the Cupric ion. Jour. Amer. Chem. Soc., 64: 1212, 1942.

5. The addition of 0.10 per cent hydrogen peroxide to washed cream contaminated with copper causes the development of oxidized flavor.

6. Under the conditions of this experiment, thiamine, pyridoxine, riboflavin, and cysteine in the presence of copper do not cause the development of oxidized flavor in washed cream.

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OXIDIZED FLAVOR IN MILK. XII. FURTHER STUDIES OF ASCORBIC ACID MECHANISMS IN THE PRODUCTION OF OXIDIZED FLAVOR IN MILK*

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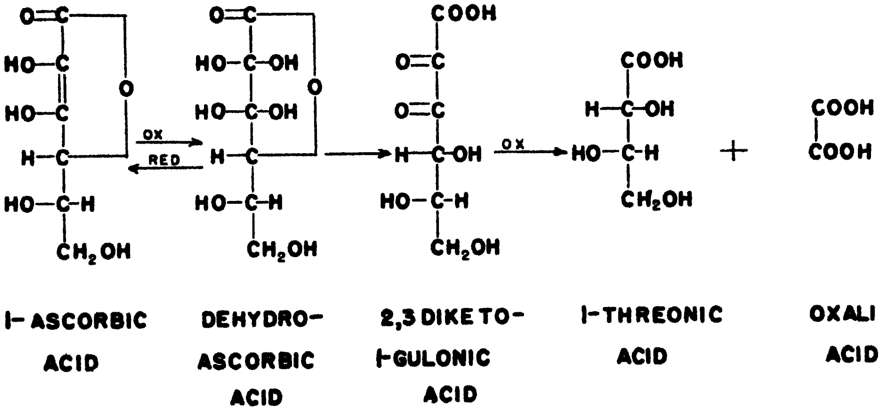
The role of ascorbic acid in the production of oxidized flavor in washed cream has been reported by Olson and Brown (8). The mechanism was ascribed to the following reactions: (a) reduction of cupric copper by ascorbic acid, which was oxidized to dehydroascorbic acid, (b) oxidation of the cuprous ion to the cupric ion by oxygen from the air with the liberation of hydrogen peroxide, and (c) oxidation of the phospholipids in the fat globule membrane of the cream by the hydrogen peroxide, producing the oxidized flavor.

Many studies have been made of the oxidation of ascorbic acid to dehydroascorbic acid, but very little information is available on the oxidation of dehydroascorbic acid.

Stotz, Harrer, and King (10) found difficulty in explaining the absorption of oxygen by copper-catalyzed ascorbic acid at a pH of 7.4. They found that about twice as much oxygen was consumed as was indicated by the indophenol titration.

Borsook *et al.* (1) made a study of the oxidation of ascorbic acid and proposed the following reactions:



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The first reaction was found to be reversible. At a pH below 4.0 the oxidation was found to stop at the dehydro form. Above pH 4.0 an irreversible nonoxidative rearrangement changed the dehydroascorbic acid to diketogulonic acid. That dehydroascorbic acid undergoes a rearrangement has been shown by a change in optical rotation by Herbert *et al.* (5). By the use of dyes, Borsook *et al.* (1) were able to determine the oxidation-reduction potential of the diketogulonic acid; they found it to be more reducing and a stronger acid than the reduced ascorbic acid. Between pH 4.0 and 9.0 the diketogulonic acid could be oxidized to oxalic acid and l-threonic acid. At a pH above 9.0 they believed the threonic acid was oxidized to tartaric acid.

Steinman and Dawson (9) studied the mechanism for the oxidation of ascorbic acid. They found that when ascorbic acid was oxidized by the cupric ion, hydrogen peroxide was evolved, but when the reaction was catalyzed by the enzyme oxidase (copper-proteinate) no hydrogen peroxide was evolved.

At the pH of milk the reactions whereby ascorbic acid and diketogulonic are oxidized are both possible. Whether or not the diketogulonic acid can be oxidized by copper with the production of hydrogen peroxide as in the reduced ascorbic acid is not known, but the fact that the diketogulonic acid is more reducing than ascorbic acid would seem to indicate this possibility. If the development of oxidized flavor in milk follows the mechanism outlined there appears to be little possibility for the action of an enzyme, although such an enzyme has already been reported.

Kende (7) reported that milk heated to 80–85° C. did not develop an oxidized flavor and attributed this to the inactivation of the enzyme "oleinase." Kende (7) and later Chilson (2) and Dahle and Palmer (3) found the heat labile substance responsible for the oxidized flavor to be associated with the milk plasma. Gould and Sommer (4) and Josephson and Doan (6) found that sulfhydryl compounds formed when milk was heated to high temperatures, acted as antioxidants and inhibited the development of oxidized flavor. Gould and Sommer (4) stated "A further proof that the enzyme theory may not be an entirely satisfactory explanation relative to heat prevention of oxidized flavor is that the same temperatures which inhibited the flavor when the copper was added prior to the heating process were of no benefit when the copper was added following heating." These results make questionable the presence of an enzyme in the development of oxidized flavor. Since it is possible to develop an oxidized flavor in washed cream; and since washed cream would contain practically no sulfhydryl groups after washing, it seemed advisable to determine if high temperature pasteurization would inhibit the development of oxidized flavor in washed cream. Accordingly, this factor was likewise studied.

EXPERIMENTAL

Milk samples were obtained from cows whose milks were susceptible to oxidized flavor development and washed cream and control milk samples for use in this experiment prepared as previously described (8).

The control sample of milk was divided into four half-pints. One of these was used as a control with no added copper while the others were contaminated with 0.5, 1.0, and 1.5 p.p.m. of copper, respectively, added from a copper sulphate solution. The washed cream was divided into three lots. Each lot was then divided and contaminated with copper in the same way as the milk. In addition to the added copper, lot one was fortified with 50 mg. of ascorbic acid per half-pint sample, which is equivalent to 210 mg. per liter. Lot two contained the same amount of ascorbic acid and sufficient KI to make a 0.1 per cent solution of the cream. Lot three was fortified with 210 mg. of dehydroascorbic acid in the same manner as lot two. The dehydroascorbic acid was prepared by oxidation of ascorbic acid with iodine solution after the method of Herbert *et al.* (5). The potassium iodide was added to lot two as a control to show the effect of potassium iodide on ascorbic acid. All samples were then stored at 40° F. ± 5° for three days, after which they were scored by at least three judges familiar with oxidized flavor. Since the oxidized flavor which developed in the washed cream was so much stronger than that developed in the control milk, no attempt was made to classify its intensity. The results of this experiment are shown in table 1. An examination of the data reveals that dehydroascorbic acid as well as the reduced ascorbic acid promotes an oxidized flavor. However, the dehydroascorbic acid produces an oxidized flavor only in a buffered solution whereas the reduced ascorbic acid will promote the flavor in either buffered or unbuffered washed cream. The addition of 0.1 per cent KI prevented the development of an oxidized flavor in the washed cream when 210 mg. per liter of ascorbic acid was present. Buffering had no effect on this reaction.

It seemed desirable to determine whether or not the concentration of dehydroascorbic acid had any appreciable effect on the development of the oxidized flavor in washed cream. Accordingly, several concentrations were selected and set up in the same manner as those reported in table 1. The results are reported in table 2. An examination of these data reveals clearly that concentrations of dehydroascorbic acid from 210 to 21 mg. per liter will, in most cases, cause the development of an oxidized flavor in washed cream contaminated with copper. All trials were made on buffered creams.

An examination of the data in table 1 reveals that an oxidized flavor was not developed in washed cream when it contained 0.1 per cent KI. Since the strongest oxidized flavors were developed at concentrations of 40 to 100 mg. of ascorbic acid per liter it seemed advisable to determine if the effect of potassium iodide in protecting against this flavor held throughout this

TABLE 1

The relationship of ascorbic acid and dehydroascorbic acid to oxidized flavor in washed cream

Date	Control milk				Washed cream												Buffered or unbuffered
					210 mgm./l. ascorbic acid				210 mgm./l. ascorbic acid + 0.1% KI				210 mgm./l. dehydroascorbic acid + 0.1% KI				
	p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	
2/2/42	–*	2*	3*	3*	ox†	ox	ox	ox					–	–	–	–	Unbuffered
2/4/42	–	2	3	3	ox	ox	ox	ox					–	–	–	–	"
2/10/42	–	2	3	3					–	–	–	–	ox	ox	ox	ox	Buffered
2/14/42	–	1	1	1					–	–	–	–	ox	ox	ox	–	"
2/17/42	–	1	1	2	ox	ox	ox	ox	–	–	–	–	ox	ox	ox	ox	"
2/19/42	–	1	1	1	ox	ox	ox	ox	–	–	–	–	ox	ox	ox	ox	"
2/19/42	–	1	1	1	–	ox	ox	ox	–	–	–	–	–	–	–	–	Unbuffered
2/21/42	–	1	2	2	ox	ox	ox	ox	–	–	–	–					"
2/24/42	–	1	2	2					–	–	–	–	–	–	–	–	"

* Intensity of oxidized flavor: – no oxidized flavor; ? doubtful; 1 slight oxidized flavor; 2 moderate oxidized flavor; 3 pronounced oxidized flavor; and 4 very pronounced oxidized flavor. † Oxidized flavor.

TABLE 2

The relationship of varying concentrations of dehydroascorbic acid to oxidized flavor in washed cream

Date	Control milk				Washed cream																Buffered or unbuffered
					210 mgm./l. dehydroascorbic acid*				105 mgm./l. dehydroascorbic acid*				42 mgm./l. dehydroascorbic acid*				21 mgm./l. dehydroascorbic acid*				
	p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	
3/20/42	–†	1†	2†	2†	ox†	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	–	ox	ox	ox	Buffered
3/21/42	–	1	1	1	–	–	–	–	–	ox	ox	ox	–	–	–	ox	–	–	–	–	"
3/23/42	–	1	2	2	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	–	ox	ox	ox	"
4/14/42	–	1	1	1	ox	ox	ox	ox	ox	ox	ox	ox	–	ox	ox	ox	–	ox	ox	ox	"
4/15/42	–	1	1	1	ox	ox	ox	ox	ox	ox	ox	ox	–	ox	ox	ox	–	ox	ox	ox	"

* Dehydroascorbic acid was prepared by oxidizing ascorbic acid with 0.1 N iodine solution and hence the solution added contained an amount of KI equivalent to the ascorbic acid added. † See table 1, footnote.

range. Accordingly, various concentrations of reduced ascorbic acid were tried in washed cream containing 0.1 per cent KI. The results of these experiments are shown in table 3. An examination of the data recorded in table 3 shows that in all cases containing 210 mg./l. ascorbic acid except two, the action of the potassium iodide was sufficient to protect the washed cream against the development of oxidized flavor. At concentrations of 40 to 100 mg. per liter of ascorbic acid, the oxidized flavor developed. This would appear to check with an earlier observation (8) that the strongest development of oxidized flavor occurred at these concentrations of ascorbic acid.

The protection of washed cream from the development of oxidized flavor by potassium iodide was unexpected. A partial explanation of this may be that the oxidation-reduction potential of hydrogen peroxide is much lower than that of potassium iodide. The standard method of determination of hydrogen peroxide is to add potassium iodide; the hydrogen peroxide liberates iodine which is then titrated with sodium thiosulfate. This, however, does not explain why potassium iodide did not protect with 40 to 100 mg. per liter of ascorbic acid. It seemed desirable to determine if potassium iodide has the same effect on milk. Accordingly, a number of experiments were planned using various concentrations of potassium iodide. Likewise it was thought advisable to determine if potassium chloride and sodium bromide had the same effect. In all these trials whole milk was used and various amounts of the salts in solution were added to give the desired percentage. The samples were stored and flavor determinations made in the same manner as the other experimental samples. The results are shown in table 4. These data reveal that 0.1 per cent KI in milk will protect against the development of metal-induced oxidized flavor. Lower concentrations give correspondingly lower protection. Concentrations of 0.1 per cent potassium chloride or 0.1 per cent sodium bromide give at most only a slight reduction in the intensity of the oxidized flavor developed.

The development of oxidized flavor in washed cream by ascorbic acid, glutathione, hydrogen peroxide, and dehydroascorbic acid, in the presence of copper, lends additional circumstantial evidence to the view that an enzyme is not involved in the development of oxidized flavor. To obtain further evidence on this point three samples of milk were obtained and treated in the same manner as the previous experimental samples except that the milk was pasteurized at a temperature not lower than 180° F. for 5 minutes prior to the washing of the cream. A sample of the raw milk was saved prior to pasteurization and served as a control sample. The results of this study are shown in table 5. Examination of the data reported in this table shows that a strong cooked flavor was found in all the samples of milk pasteurized at 180° F. after three days. However, in the washed cream a strong oxidized flavor was developed in all cases. Even when copper was not added, the presence of the ascorbic acid in the washed cream

TABLE 3

Relationship of varying concentrations of ascorbic acid to oxidized flavor in washed cream containing 0.1% KI

Date	Control milk				Washed cream																Buffered of unbuffered
					210 mgm./l. ascorbic acid				105 mgm./l. ascorbic acid				42 mgm./l. ascorbic acid				21 mgm./l. ascorbic acid				
	p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	
2/10/42	–*	2*	3*	3*	–	–	–	–													Buffered
2/14/42	–	1	1	1	–	–	–	–													Buffered
2/17/42	–	1	1	2	–	–	–	–													Buffered
2/19/42	–	1	1	1	–	–	–	–													Unbuffered
2/21/42	–	1	2	2	–	–	–	–													"
2/24/42	–	1	2	2	–	–	–	–													"
2/28/42	–	1	1	2									ox	ox	ox	ox	ox	ox	ox	ox	Buffered
3/4/42	–	1	2	2	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	"
3/7/42	–	1	2	2	–	–	–	–	ox	ox	ox	ox	ox	ox	ox	ox	?	–	–	–	Unbuffered
3/9/42	–	1	2	2	–	–	–	–	ox	ox	ox	ox	ox	ox	ox	ox	–	–	–	–	"
3/10/42	–	1	1	1	–	–	–	–	ox	ox	ox	ox	ox	ox	–	–	ox	–	–	–	"
3/12/42	–	1	1	2	–	–	–	–	ox	ox	ox	ox	ox	ox	ox	ox	–	–	–	–	"
3/13/42	–	1	2	2	–	–	–	–	ox	ox	ox	ox	ox	ox	ox	–					"
3/19/42	–	1	2	2	ox	ox	ox	–	ox	ox	ox	–	ox	ox	ox	ox	ox	ox	ox	ox	Buffered

* See table 1, footnote.

TABLE 4

Relationship of varying concentrations of potassium iodide to oxidized flavor in milk

Date	Control milk				Milk + 0.1% KI				Milk + 0.05% KI				Milk + 0.01% KI				Milk + 0.1% KCl				Milk + 0.1% NaBr			
	p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper			
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5
2/21/42	–*	1*	2*	2*	–	–	–	–																
2/23/42	–	1	2	2	–	–	–	–									–	?	1	2				
2/24/42	–	1	2	2	–	–	–	–													–	?	1	2
2/25/42	–	1	1	1	–	–	–	–					–	?	1	1					–	?	?	1
2/27/42	–	1	1	2	–	–	–	–					–	1	2	2					–	–	?	1
3/3/42	–	1	1	2	–	–	–	–	–	–	?	1	–	1	1	2	–	–	?	1	–	–	?	1
3/4/42	–	1	2	2	–	–	–	–	–	–	?	1	–	?	1	1	–	1	1	1				
3/14/42	–	1	1	1	–	–	–	–	–	–	–	1	–	?	1	1								

* See table 1, footnote.

TABLE 5

The relationship of high heat on milk to the development of oxidized flavor in washed milk

Date	Milk								Washed cream								
	Control				180° F.–5 min.				210 mgm./l. ascorbic acid				105 mgm./l. ascorbic acid				Buffered or unbuffered
	p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	
2/11/42	–*	2*	2*	3*	– H.H.†	– H.H.	– H.H.	– H.H.	ox	ox	ox	ox					Unbuffered
3/2/42	–	1	1	2	– H.H.	– H.H.	– H.H.	– H.H.					ox	ox	ox	ox	"
3/23/42	–	1	2	2	– H.H.	– H.H.	– H.H.	– H.H.					ox	ox	ox	ox	Buffered

* See table 1, footnote.
† H.H.—High heat or cooked flavor.

caused the development of the flavor in the limited number of cases studied. As enzymes are inactivated at high temperatures, these data show quite clearly that an oxidized flavor can be produced chemically without the aid of any enzyme and give further evidence that no enzyme is involved in the production of an oxidized flavor.

DISCUSSION OF RESULTS

The results of this experiment indicate that both ascorbic acid and dehydroascorbic acid are capable of producing an oxidized flavor in washed cream. In the case of dehydroascorbic acid it is necessary that the washed cream be buffered to control the pH since the flavor does not develop in strongly acid solutions. A review of the literature indicates that theoretically both the reduced ascorbic acid and the dehydroascorbic acid are capable of causing the development of oxidized flavor. This was found to occur under the conditions of this experiment. Likewise, it was found that the addition of 0.1 per cent potassium iodide to milk was sufficient to prevent the development of an oxidized flavor and that lesser amounts inhibited the flavor development to a lesser degree. Neither potassium chloride, nor sodium bromide were found to be as effective as potassium iodide. No complete explanation for this effect is evident at the present time.[1]

A study of high temperature pasteurization reveals that the sulfhydryls are contained in the plasma portion of the milk and are removed sufficiently when cream is washed so as to render the cream susceptible to the development of oxidized flavor with ascorbic acid. This would indicate that no enzyme is involved in the production of an oxidized flavor.

CONCLUSIONS

1. Washed cream fortified with ascorbic acid (210 mg. per liter) and contaminated with copper develops a strong oxidized flavor.

2. Washed cream fortified with dehydroascorbic acid (40 to 200 mg. per liter) and contaminated with copper develops a strong oxidized flavor when buffered.

3. When washed cream containing 210 mg. of ascorbic acid per liter is contaminated with copper the development of oxidized flavor is prevented in most cases by a 0.1 per cent concentration of potassium iodide.

4. When contaminated with copper, susceptible milk containing 0.1 per cent potassium iodide does not develop an oxidized flavor. Lesser concentrations of potassium iodide are ineffective in most cases.

5. Pasteurization of milk at 180° F. for 5 minutes prior to washing of the cream does not affect the susceptibility of the cream to oxidized flavor when contaminated with copper.

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[1] Since submitting this article for publication, Mapson, (Biochem. Jour. 35: 1332–53, 1941) has shown that halides have a protective action on the oxidation of ascorbic acid in the order I > Br > Cl. He attributed the protective action to a copper halide complex ion.

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THE EFFECT OF OXYTOCIN ON MILK AND MILK FAT SECRETION[1]

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It was demonstrated previously that there was a marked decrease in the uptake of blood fat by the mammary gland immediately following milking, after which the fat utilization gradually increased (2). That the distension of the alveoli was necessary for the normal passage of fat into the gland was indicated by a number of experiments in which one half of the udder was milked out and the other half left distended with milk; arterio-venous blood samples for fat analysis were then drawn simultaneously from both sides and it was found that while the distended half used the normal amount of blood fat, the half milked out used little or no blood fat. The actual removal of the milk from the gland and not the "letting down" of milk was therefore responsible for the decrease in the utilization of blood fat.

From the above it appeared that by removing the milk from the gland at frequent intervals it should be possible to retard the passage of blood fat into the gland sufficiently to decrease the amount of fat secreted by the gland. Data are presented in this paper on the results of such a study, in which oxytocin was used to get complete ejection of milk at each milking. In addition, since oxytocin is being used as an aid in obtaining milk under certain experimental conditions, as in the case of the perfused gland (1), a study was made of the effect of oxytocin upon normal milk and fat production when injected at regular milking intervals.

EXPERIMENTAL

In the following studies obstetrical pituitrin was used to obtain complete ejection of milk from the gland and will be referred to as oxytocin.

The effect of oxytocin injections at regular milking intervals is shown in figure 1. The total milk and fat production is shown during a preliminary period of seven days, during a nine-day experimental period, and for seven days following the experimental period. During the nine-day experimental period the cow was milked normally twice each day. Immediately following each milking 10 I.U. of oxytocin were injected intravenously and the residual milk obtained. The milk drawn normally and the residual milk obtained with oxytocin were tested separately for fat. It will be noted that with the exception of the first day of oxytocin injection and the day after

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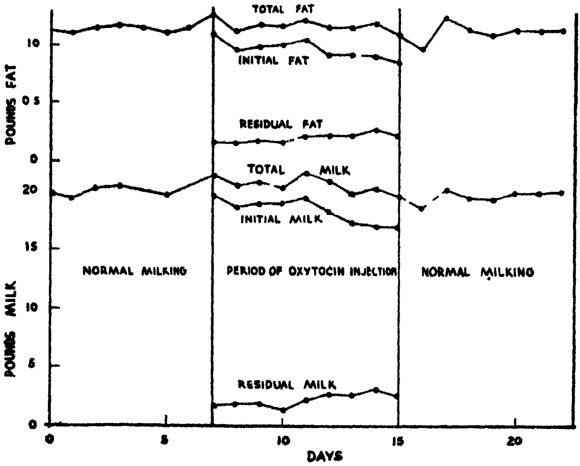

FIG. 1. The effect of oxytocin injections at regular milking intervals upon milk and milk fat production.

the last injection of oxytocin the total milk and fat production was not significantly affected by the oxytocin. The increased production of milk and fat on the first day was due entirely to the residual milk obtained with the first injection of oxytocin. The drop in production on the day following the cessation of oxytocin injection was due to the failure to obtain the residual milk on the first milking following the last injection of oxytocin. The amount of milk and fat actually secreted during the period, therefore, was not significantly changed by the use of oxytocin. During the nine-day period of oxytocin injection the normally drawn milk was lower in fat than during the control period, testing 5.24 per cent as compared to a normal of 5.72 per cent. However, the residual milk averaged 9.45 per cent so that the total milk obtained during the period of oxytocin injection did not differ significantly in fat per cent (5.69) from the milk obtained during the control

TABLE 1

Effect of milking at short intervals with the aid of oxytocin upon total milk and milk fat production

(Average of four cows)

	Milking interval	Length of period	Milk production	Fat production	Per cent of fat in milk
			lbs.	*lbs.*	
Preliminary period (normal milking)	12 hours	24 hours (3-day average)	26.65	1.23	4.62
Experimental period (10 I.U. of oxytocin at each milking)	2 hours	24 hours	26.22	1.06	4.04

period. During the period of oxytocin injection the first drawn or "initial" milk and fat decreased but was compensated for by an increase in the amount of residual milk and fat.

Four cows were milked at intervals of two hours for periods of from 30 to 40 hours. Ten I.U. of oxytocin were injected intravenously preceding each milking. The data are summarized in table 1. The first two milkings are not included in the averages as they were usually abnormally high in fat content due to the residual milk and did not represent the actual secretion during the period of oxytocin injections. The average production of the four cows during a 24-hour period in which the cows were milked every two hours is compared with the average daily production of these cows during a preliminary period of three days.

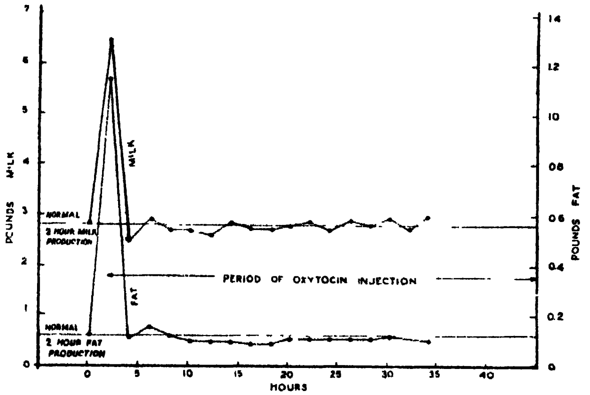

FIG. 2. Milk and milk fat production as affected by milking at two hour intervals with the aid of oxytocin injections.

It will be observed in table 1 that while there was no marked reduction in the amount of milk produced during the experimental period, there was a decrease in the average fat production. This decrease occurred in each of the four cases under observation and averaged 13.8 per cent, whereas total milk production decreased only 1.6 per cent.

The detailed data on one of the animals, which is typical of the group, are presented diagrammatically in figure 2. The milk and fat production at two-hour intervals over a period of 34 hours is compared with the expected two-hour production on the basis of the previous three-day production. Following the first two injections of oxytocin the milk production at two-hour intervals varied less than seven per cent from the expected two-hour production in a period of 30 hours. The total milk production in the 30-hour period was identical with the rate of milk secretion during the three-day preliminary period. The fat production during the 30-hour period was 11.0 per cent less than the rate of fat production during the three-day preliminary period.

The various fat constants were determined on the butterfat obtained from the animal represented in figure 2 at four intervals during the 34-hour period, a composite being made of every four milkings in order to obtain sufficient fat for analysis. The Polenske and saponification values were not affected. The Reichert-Meissl value decreased during the first eight hours from 31.7 to 28.5 and remained at that level. The iodine value decreased from 30.1 to 21.6 during the first eight-hour period, declined to 21.3 during the second eight-hour period, and then increased to 27.8 and 28.5, respectively, during the next two eight-hour periods. The iodine values were also determined on the fat samples from two of the remaining three animals. In one case there was no appreciable change, while in the other there was a decline within 10 hours from 40.1 to 37.8. The reason for these variations is not apparent.

DISCUSSION

The finding that oxytocin does not inhibit milk secretion is not in agreement with the conclusion reached by Turner and Slaughter (3). However, the data presented by these workers are believed to indicate incomplete milking following the injection of oxytocin, due partially to the storage of residual milk during the subsequent milkings, rather than inhibition of milk secretion. A certain amount of residual milk is always present in the mammary gland following normal milking. When this milk is removed by the use of oxytocin the next milking is usually deficient by that amount unless oxytocin is again used, as some of the milk normally obtained at that time will be stored as residual milk.

Milking with the aid of oxytocin at intervals of two hours demonstrated that the rate of milk secretion is extremely constant and is not influenced by the removal of the milk from the gland. The decrease in milk fat secretion when the gland was milked out at short intervals can be explained on the basis of previous work (2), showing that the removal of milk from the gland decreases the uptake of blood fat. However, the decrease was not as large as might be expected on the basis of this work. Possibly the storage of fat within the glandular tissue was sufficient to supply most of the fat needed for normal fat secretion.

CONCLUSIONS

1. The injection of oxytocin at regular milking intervals did not affect the normal milk or fat secretion.

2. Milking at two-hour intervals with the aid of oxytocin did not affect total milk production but decreased milk fat secretion 11.0 per cent in 24 hours, probably by inhibiting the passage of blood fat into the glandular tissue.

3. The rate of milk secretion from hour to hour is comparatively constant and is not changed by the removal of milk from the gland at regular milking intervals or at shorter intervals of two hours.

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THE ERADICATION OF BOVINE TUBERCULOSIS*

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THE FIRST TWENTY-FIVE YEARS OF THE EFFORT

The attempt to eradicate tuberculosis from the cattle of this country took definite shape in 1917. It has touched most intimately the producers of milk since from their herds great numbers of cattle have been removed for slaughter. It has also been of interest to all those using milk as a raw material in dairy manufacturing since the healthfulness of the products is involved. It was thought that a review of the accomplishments of the quarter century and the indication of some of the conditions revealed in the work might be of interest to students of dairying.

On December 31, 1940, the Secretary of Agriculture made, in a radio address, the following statement: "Twenty-three years ago veterinarians believed as a result of research that tuberculosis of cattle could be stamped out in this country. This year *their* belief was *completely* vindicated. Tuberculin testing of cattle has now been completed in every county. The United States is *now practically free* of *bovine tuberculosis.*"

The Secretary of Agriculture was referring to the fact that in 1940 the entire country had reached the modified accredited status, *i.e.*, an area, usually a county, in which less than 0.5 per cent or five per 1000, of all animals tested react positively to a diagnostic dose of tuberculin.

The period of research to which the Secretary indirectly refers started in 1893–94, when the first tuberculin tests were made in this country. The research showed the value of tuberculin as a diagnostic agent for tuberculosis in the bovine and it also yielded the intradermal test without which the country-wide testing would have been impossible, since the original subcutaneous test would have been prohibitorily expensive. The period was also one of education of farmers as to the economic significance of the disease, of medical men as to the probable relation of bovine tuberculosis to tuberculosis in man, and of the general public to the same realization. Thus by 1917 the public was in sympathy with the use of public funds for the compensation of farmers whose cattle were found tuberculous. The farmers, having been convinced of the continued tax the disease placed on them, were, in large part, willing to submit their herds to examination and to bear a part of the loss incurred from the slaughter of reacting animals. An appropriate tool for diagnosis was available. Thus the stage was set for the great co-

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operative endeavor by the Federal Government, represented by the Bureau of Animal Industry, and by the various state agencies responsible for livestock sanitation.

The effort is unquestionably the world's greatest in the field of disease eradication in the case of the domestic animals and possibly in all disease eradication. The plan was well conceived and the effort has been well sustained for a quarter of a century.

It seems proper to now review the work, to evaluate the results, and to view the problems of the future, since the task is not completed. The italicized words in the quotation from the Secretary of Agriculture appear underlined in the official mimeographed release of the broadcast. It is certain that no one familiar with the work believes that the disease is completely eradicated, but rather believes that much effort and money must still be expended before the "mopping up" is completed. The extent and nature of the "mopping up" operations are questions of present significance.

TABLE 1

Tuberculin testing under accredited-herd and area plans, 1917–41

Year ended June 30	Cattle tested	Reactors found and removed	
	Number	*Number*	*Per cent*
1917	20,101	645	3.2
1918	134,143	6,544	4.9
1919	329,878	13,528	4.1
1920	700,670	28,709	4.1
1921	1,366,358	53,768	3.9
1922	2,384,236	82,569	3.5
1923	3,460,849	113,844	3.3
1924	5,312,364	171,559	3.2
1925	7,000,028	214,491	3.1
1926	8,650,780	323,084	3.7
1927	9,700,176	285,361	2.9
1928	11,281,490	262,113	2.3
1929	11,683,720	206,764	1.8
1930	12,845,871	216,932	1.7
1931	13,782,273	203,778	1.5
1932	13,443,557	254,785	1.9
1933	13,073,894	255,096	2.0
1934	15,119,763	232,368	1.5
1935	25,237,532	376,623	1.5
1936	22,918,038	165,496	0.7
1937	13,750,308	94,104	0.7
1938	14,108,871	89,359	0.6
1939	11,186,805	60,338	0.5
1940	12,222,318	56,343	0.5
1941	12,229,499	40,702	0.3
Total	241,943,522	3,808,903	1.6

The incidence of the disease revealed by the country-wide test was not as high as had been predicted from the preceding tests made in the areas in which, because of the length of time involved and the frequency of change in the herds by sale and purchase of animals, the disease had had much greater opportunity to spread than in the usual herd or area. The findings emphasize the slowness with which bovine tuberculosis spread in the early years of the cattle industry of the country and indicate that a rapid increase from still unrevealed foci will not occur and that the apprehension of some regarding the recrudescence of the disease is not well founded. The record of Marathon County, Wisconsin, is evidence of this view. The dairy industry was well established therein by the beginning of the century, but when the first complete test of the 103,000 cattle was made in 1926, the incidence of reactors was but 0.53 per cent, a level not much above that of the entire country after 25 years of intensive effort.

The complete record of the eradication effort is presented in table 1, taken from the report of the Chief of the Bureau of Animal Industry for 1941. It should be remembered in connection with the data as to reactors that much of the testing in 1917–20 was in areas in which much testing had been done before 1917. It is estimated that nearly $300,000,000 of public funds have been spent in the effort, to which must be added the cost to the farmer. If one can judge from the experiences of England, in which the incidence of tuberculosis in cattle is over 40 per cent, the expenditure made is well worth while since, without the eradication program, the incidence in this country would have undoubtedly reached the English level at some future time. Indeed it had done so in certain areas in this country by 1920. The present generation has, in reality, purchased health and insurance against economic loss for future generations as regards bovine tuberculosis.

Certain states reached the stage characterized by the Secretary of Agriculture as practically free of bovine tuberculosis over a decade ago; others more recently. The data regarding these states are presented in Table 2, compiled from the monthly reports of the Bureau of Animal Industry. This record is that after the date of accreditation and should give some preview of the future record.[1] The record of certain states and counties shows that the time and effort demanded to reduce the incidence of reactors from a very high level to a low one is much less than to reduce the incidence from a low level to one falling much below the modified accredited level of 0.5 per cent.

The data for New York, accredited in 1935, and for California, accredited in 1940, are as follows:

NEW YORK

	Animals tested	*Reactors per 1000 tested*
1930	1,199,987	43.0
1934	1,938,579	80.0
1936	2,075,444	5.4
1940	1,363,237	4.9
1941	1,291,207	3.9

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[1] The data for 1937 are not given since the record of that year was not available to the writer.

CALIFORNIA

	Animals tested	*Reactors per 1000 tested*
1935	961,121	86.0
1936	1,586,769	35.0
1940	1,571,313	6.8
1941	1,401,230	6.4

The same point is made evident by the record of two counties in Wisconsin.

KENOSHA COUNTY

	Year	*Reactors per 1000*
Original complete test	1927	398.0
Second complete test	1929	6.6
Third complete test	1932	7.7
Fourth complete test	1934	5.6
Fifth complete test	1937	1.0

MARATHON COUNTY

Original complete test	1926	5.3
Second complete test	1929	1.8
Third complete test	1932	1.9
Fourth complete test	1938	0.8

The states, the records of which are presented in table 2, represent both the north and the south, great dairy states such as Wisconsin and Michigan in which the herds are large and in which the cattle are stabled for a large part of the year, and other states in which the herds are small, the animals stabled only for short periods and probably the herds less subject to change by sale and purchase than the herds of the intensive dairy areas of the north. The sample used in table 2 would seem to be a fair one of the entire country and the record of the states included in table 2 one which will later be duplicated by other states.

In 1917 the dominant idea, indeed the only idea, was that the sensitization of the bovine to tuberculin was always occasioned by the bovine tubercle bacillus, the organism that can and does cause progressive and fatal tuberculosis in the bovine. It was, therefore, concluded that every reacting bovine is a focus from which tubercle bacilli are passing or may, at some future time, pass to the bovine associates of the reacting animal, to swine and to humans. The removal of every reacting animal for slaughter was, therefore, fully justified, and as is shown in table 1, 3,808,903 animals were removed and slaughtered in the period 1917–1941 inclusive, a number equal to about 7 per cent of the total number of cattle in the country at any one time.

This supposition had another implication; namely, a reasonable hope that, within a period of years, all sources of infection would be removed, the foci from which the organism could pass to still healthy cattle would no longer exist, and no further reactors would be found. The end of the road

TABLE 2

The incidence of animals reacting to tuberculin expressed in units per 1,000 units tested

	1929	1930	1931	1932	1933	1934	1935	1936	1938	1939	1940	1941
North Carolina	1.9	1.2	1.6	2.4	1.5	3.2	1.8	0.3	0.1	1.2	0.03	0.22
Maine	4.4	4.0	3.3	4.0	2.7	1.4	2.6	1.3	1.3	1.0	0.9	0.63
Michigan		4.6	3.7	1.7	1.3	2.2	1.6	1.3	0.9	1.8	2.3	2.3
Indiana			5.2	4.8	5.8	5.4	3.6	3.4	3.0	2.5	2.0	1.5
Kentucky						2.9	1.5	1.3	0.6	2.7	3.0	1.8
West Virginia						1.4	1.6	1.1	2.8	1.3	2.3	1.4
Washington						7.0	5.6	4.1	5.4	2.0	1.5	2.0
Wisconsin		3.8	3.2	5.0	6.1	2.6	1.9	2.1	1.9	2.9	1.8	1.7

would have been reached. The only obstacle in the way seemed to be the tuberculous animal, the skin of which was not sensitized to tuberculin by the growth of the bovine tubercle bacillus in its tissues, or again the animal the tissues of which had been made insensitive to tuberculin, a condition possibly obtaining in far advanced cases of the disease. The latter type should pass from the herd by death or sale as a discard; the first type might continue in the herd, but not for a period much longer than a cow generation, which is, in most dairy areas, not much over six years. The ease with which the incidence of bovine tuberculosis was reduced from a very high level to a low one is evidence that this type of animal cannot be an important factor in the persistence of reactors, the phenomenon illustrated by the data of table 2.

Other evidence of the hope that a definite stopping place would be reached was the attitude toward the reacting animal that showed no evidence of tuberculosis on post-mortem examination. The expression first used to refer to such animals was "no-lesion"; later changed to "no-visible-lesion." The latter seemed proper since it was thought that all such were tuberculous, but that for one reason or another the lesions were not detected. Evidence slowly accumulated to show that the matter was not so simple as had been earlier thought. In those areas in which the disease had reached a high level of incidence, one would expect to find only few of the no-visible-lesion animals among the reactors, while in the areas of low incidence they would form a larger part of the reacting group. In the repeated tests of the cattle of any area, the foci from which bovine tubercle bacilli could spread were being removed. Indeed the evidence points to very complete removal of such foci. This would tend toward the elimination of the type of cases thought earlier to be responsible for the no-visible-lesions.

If, however, one admits that a bovine may be sensitized to tuberculin by some relative of the bovine tubercle bacillus not able to produce a progressive type of disease, the record presented in table 2 seems to make sense, but does not make sense on the basis of the prevalent ideas of 1917. The incidence of the reactor in Wisconsin in 1941 is not much less than in 1935; in Michigan the value for 1941 is greater than that for 1932. The records of the other states seem to offer more hope of reaching the final goal of no reactors, no more testing, and no more expense to government or to individuals. Now and then the record of a state indicates the near approach to the desired goal of no reactors. Thus, in Arkansas in the 3-year period, September 1938 to October 1941, no reactors were found in approximately 55,000 cattle. In October 1941 one reactor was found, and in November two. In other states a greater number of animals have been tested without a reactor than in Arkansas, but in most cases the goal seems from the present record unattainable, at least if present methods continue to be used. The desired goal is freedom from tuberculosis caused by the bovine tubercle bacillus, not what was once thought to be the same goal, no reactors to tuberculin.

All now agree that the avian tubercle bacillus can and does sensitize bovines to the usual tuberculin used in this country, made with the human tubercle bacillus. It is evident that if the goal of no reactors is to be reached, tuberculosis in farm poultry must be eradicated. The report of the Bureau of Animal Industry for 1941 states that avian tuberculosis was found on 22 per cent of 11,454 farms inspected in the midwestern and north central states. The disease in poultry can be quickly and cheaply eradicated by proper management of the flocks.

In most areas some of the reacting cattle show no internal lesions of tuberculosis, but do show lesions on the skin of the legs in which acid-fast bacilli can be found on microscopic examination. Earlier it was thought these were true bovine tubercle bacilli, but the inability to cultivate them on artificial media in numerous trials extending from 1930 to the present indicates that the earlier idea is no longer tenable. There is no reason to believe that this unknown organism is able to cause serious trouble in the infected animal, or is of significance in other relations. The ability of the organism to sensitize the animal to tuberculin and its resistance to cultivation indicate the possibility that other similar organisms may occur in other regions of the body.

The human tubercle bacillus has been emphasized as a possible sensitizing agent for cattle. Observations made both in the field and under experimental conditions in Finland indicate that this agent is a disturbing factor in the attempted eradication of bovine tuberculosis in that country. There the final stages in the effort seem to be quite similar to those appearing in certain areas of this country. The conditions existing in this country are so different from those of Finland that even though the human tubercle bacillus may be significant in relation to the sensitization of cattle in Finland, it is not likely to be of like importance here.

The acid-fast organism causing Johne's disease in cattle and in sheep may also prove to be a confusing factor of significance as may other members of the group of bacteria of which the tubercle bacilli are members. Such organisms are widely distributed in nature, especially in soil, on plants and in and on the animal body. Their constant occurrence in the fatty secretions of man is a well-known fact.

The sensitization of man by other than the human and bovine tubercle bacilli, the forms pathogenic for man, is being recognized by the physician using the tuberculin test. He views the test as a sieve by which he can divide any group into two parts: the nonreactors which he can dismiss and the reactors which must be examined by x-ray and by other methods in order to determine whether the reacting individual is actually tuberculous at the moment, or that the present sensitization is due to a past infection with human or bovine tubercle bacilli that has been overcome or to some other and possibly harmless organism.

The present record of those states that reached the modified accredited status some years ago indicates that the record of the future will be much the same and that there is little hope of reaching the goal, *i.e.*, the time when no reactors would be found, which in 1917 seemed possible. It seems that some reactors will continue to appear and that testing must be continued.

The question of continued slaughter of cattle is more difficult to answer. This should and must continue so long as seems necessary to safeguard the position won in the 25-year battle. The answer as to what is necessary to safeguard the present position cannot be given from present knowledge. There is no question but that some part of the cattle now removed for slaughter could be left in the herds without harm to the herd since the sensitization is due to the avian or to the human tubercle bacillus, or to some not yet cultivated member of the group. It is to be remembered that no one of these organisms is capable of causing death of the bovine or of reducing its productive capacity or is of importance as regards the healthfulness of milk, since the organisms would not be excreted, even if present in the tissues.

The fact that at least one of the agents sensitizing cattle to tuberculin has, to the present, resisted cultivation indicates that there may be others of like nature in the picture. The possibility also exists that the pathology of the infection may be diffuse rather than definite, thus making a microscopic examination for the organisms difficult. If the skin-lesion infection was diffuse rather than definite, it might not have been detected. These possibilities indicate the need of expanding the research in this field by other methods than have been commonly used; namely, gross and microscopic pathology and cultural methods either in vivo with experimental animals or in vitro. Feldman has recently reported his findings on 101 reacting animals removed from 99 herds. They indicate that 77.2 per cent were not infected with the bovine tubercle bacillus. Even those that had been classed as showing lesions on post-mortem examination were in 12 out of 44 cases infected with a sensitizing agent other than any of the types of tubercle bacilli.

The reacting animal itself must be studied as to its future conduct in the environment in which it was discovered or in a changed environment. It must be kept to write its own record. Will it continue to react if removed to another environment, thus breaking the connection between the animal and the source of the sensitizing agent, which must be constantly supplied if the sensitization is necessarily to persist? Thus, cattle can apparently be kept sensitive to the avian tubercle bacillus only by constant association with tuberculous birds, and it has even been shown that some humans that have recovered from tuberculosis lose the sensitivity to tuberculin when they are not constantly associated with other tuberculous individuals, and retain it when the association is continued. The greatest information would be gained by allowing all reacting animals to remain in the herds when a definite history of infection with the bovine type is not established. Such trials must be on an experimental basis in certain selected areas. The plan involves research on the farm and of course must provide for the payment of all costs and damages suffered by the owner of the herd. The trials should be sufficiently numerous and comprehensive to indicate the relative safety of using the information thus secured to contradict that supplied by the tuberculin test in much the same manner as the radiologist uses the information gained by x-ray or fluoroscope to confirm or to contradict the tuberculin test in man. The reports as to the number of cattle tested each month in the different states, and as to the number of instances in which "no reactors found" supply data indicating the rate of progress toward the goal of "no reactors."

	Trials	*Trials in which no reactors found*	
1938	601	78	13 per cent
1939	591	80	13 " "
1940	594	92	15 " "
1941	593	94	15 " "

Thus, out of 601 trials no reactors were found in 78 instances in 1938. The data indicate the slowness of the progress toward the goal of no reactors. Indeed, whatever progress is indicated by these data may be offset by the reduction in number of animals tested in any area in any month. Reactors are more likely to be found if many animals are tested than if few are tested. There has been a marked decrease in the frequency of testing all the cattle in certain areas. Thus, in Wisconsin there was a six-year interval between the third complete test and the fourth in the case of Marathon County and over four years between the fourth complete test in Kenosha County and the fifth. There seems to be no question concerning the wisdom of gradually lengthening the interval between examinations of all cattle in any area.

The hesitancy of those in control of programs related to human and animal sanitation toward the modification of these programs is commendable, and it is probably too much to expect that modification will be suggested by those actively engaged in the effort. Every such program at some time requires adjustment to the new conditions revealed by the program in action. Thus the program related to yellow fever is not now that of a decade ago because the old program in action revealed that yellow fever is not a disease of man alone transmitted by a specific mosquito alone, but that the causal organism has other foci than man and other vectors than the specific mosquito. The old must be supplemented or modified by the new and unless this is done in the field of bovine tuberculosis, it seems that the next decade will see the possibly unnecessary expenditure of public money and the possibly unnecessary slaughter of cattle. The situation demands, as Feldman[2] suggests, the re-examination of the question as to the possibility of other agents than tubercle bacilli sensitizing cattle to tuberculin.

[2] Amer. Jour. Vet. Res., 3: 3–9, January, 1942.

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ABSTRACTS OF LITERATURE

BOOK REVIEW

394. Outlines of Food Technology. HARRY W. VON LOESECKE. Reinhold Publishing Corporation, 330 W. Forty-Second Street, New York City. 15 chapters plus index, 485 pages; $7.00.

This book was intended as and is an outline of the more important processes for preparing and preserving food products. While extended specific details of processing have been omitted, in most cases the prevailing important steps are cited. An excellent selected list of recently published references covering details of processing is provided with each chapter. The authenticity of the subject material is bolstered in that chapters were reviewed by authorities in food processing problems, represented mostly by members of the U. S. Dept. of Agriculture of which the author is a member. Chapters of greater interest to the dairy industry profession are: The Tin Can and Glass Container, their manufacture, handling, sizes and application, defects, problems; Fruits and Their Products, including selection, preparation, processing and packing of some 70 fruit products; Dairy Products, including eggs; Edible Fats and Oils, including a short review on oleomargarine manufacture; Sugars and Starches; Nuts; Spices, Relishes and Essential Oils; Beverages, including cocoa, coffee and carbonated products and their flavoring materials. For many natural products the source of origin and characteristics are discussed. Other chapters are Canning of Vegetables; Meat, Meat Products and Poultry; Fish and Shellfish; Grains and Their Products; Confectionery, Jams, Jellies, Preserves, including pectins; Storage and Marketing of Fruits and Vegetables; Preservation of Foods by Freezing. The chapter on dairy products does not, in view of the object of the text, provide those in the dairy industry with information not already available in standard dairy texts. Mention is not made of the continuous process of casein manufacture, nor of the production of dried whey. A few sections of the book are illustrated with excellent photographs and a number of diagrams and drawings are used. The book is very readable, and descriptions of products and processes are clearly presented. The real value of this book for those in the dairy industry, is in the clear, concise information of food products used in the manufacture of the many dairy products, and similarly the nature of some food products processes in which dairy products are employed. The text will be a useful reference book for dairy and food laboratories and plant executives.

K.G.W.

BACTERIOLOGY

395. Preliminary Bacteriological Study of Market Creams. ELIZABETH D. ROBINTON, EARLE K. BORMAN, AND FRIEND LEE MICKLE, Bur. of Labs., Conn. State Dept. Health, Hartford, Conn. Jour. Milk Technol., *4*, No. 5: 253. Sept.–Oct., 1941.

In a survey made on 523 samples of market cream (both raw and pasteurized) using the direct microscopic group count and the agar plate count using incubation temperatures of 37° C. for 48 hours, 8° C. for 4 days and 55° C. for 48 hours, it was found that 45.6% of the samples failed to meet tentative standards (500,000 groups or colonies per ml.) by the direct microscopic count, 18.3% by the agar plate count incubated at 37° C. for 48 hours, 24.4% by the agar plate count incubated at 8° C. for 4 days, and 1.9% by the agar plate count when incubated at 55° C. for 48 hours.

From the results obtained it was concluded that more attention should be paid to the direct microscopic count, and that the standard agar plate count incubated at 37° C. for 48 hours should be supplemented by incubation at 8° C. for four days. L.H.B.

396. Sources of Hemolytic Enterococci Found in Milk. GEORGE E. TURNER AND F. R. SMITH, Div. Dairy Indus., Univ. Calif., Davis. Jour. Milk Technol., *4*, No. 4: 183. July–Aug., 1941.

In a study made to determine the possible sources of hemolytic enterococci, it is reported that the organisms were isolated from cow feces, water, soil and from the udder of an apparently normal cow. L.H.B.

397. A New Microscopic Procedure for the Detecting and Locating of the Source of Thermoduric Organisms in Milk. W. L. MALLMAN AND C. S. BRYAN, Mich. Agr. Expt. Sta., East Lansing, AND WILLIAM K. FOX, Dept. Health, Lansing, Michigan. Jour. Milk Technol., *4*, No. 4: 195. July–Aug., 1941.

A comparative study of the direct microscopic count with the agar plate count indicates that the direct microscopic count can be used to good advantage in determining the presence of thermoduric organisms in milk.

Incubation of the milk at 58° to 60° C. for two hours destroys the nonthermoduric organisms and causes their dissolution, leaving only the viable thermoduric organisms.

The proposed test is as follows:

1. "Place 5 to 10 ml. samples of milk suspected of containing thermoduric bacteria in an incubator of 58° to 60° C. Incubate for two hours. 2. Make a microscopic count following standard procedure. 3. Samples showing bacteria counts of 40,000 or more bacteria per ml. contain thermoduric bacteria in excessive numbers." L.H.B.

398. Inhibition of Micro-organisms by a Toxic Substance Produced by an Aerobic Spore-Forming Bacillus. H. KATZNELSON, Sci. Serv., Canad. Dept. Agr., Ottawa. Canad. Jour. Res., *20,* No. 3: Sec. C, 169. Mar., 1942.

A thermostable, diffusible substance produced by an aerobic spore-forming bacillus in potato dextrose medium was found to inhibit the growth of 77 out of 81 species of fungi. The majority of streptococci, staphylococci, bacilli, lactobacilli and clostridia tested were suppressed. Gram negative organisms were unaffected. Soil, bentonite and activated charcoal adsorbed the toxic substance. The agent passed through cellophane, parchment and collodion, resisted autoclaving for 30–45 minutes at 15 lb., but was rapidly destroyed by heating in alkaline and less rapidly in acid solutions. The substance has not yet been identified. O.R.I.

399. Requisites for the Recognition of the Blue-green *Pseudomonas.* M. C. JAMEISON, Univ. Manitoba, Winnipeg. Sci. Agr., *22,* No. 7: 401. March, 1942.

Nutrient media, made with distilled water as stipulated by certain manuals, were found inadequate for the production of pigment characterizing bacteria of the *Pseudomonas* genus. This leads to inaccuracy and delay in the identification of these types. Tap water favored pigment production and investigation showed that this was due to its content of SO_4, PO_4 and Mg. A nutrient medium was therefore formulated which allowed pigment production and increased the ease of recognizing *Pseudomonas* organisms.

An additional valuable aid in this recognition was the utilization of the property of fluorescence possessed by these types. Examination of colonies under strong purple and ultraviolet light was employed. Subsurface colonies must be allowed access to air before they show fluorescence, which may be easily secured by puncturing the agar with a sterile needle. O.R.I.

BUTTER

400. Distribution of Salt in Butter. A Volumetric Micromethod. C. L. OGG, I. B. JOHNS, W. H. HOECKER, AND B. W. HAMMER, Ia. State Col., Ames, Ia. Ind. Eng. Chem., Analyt. Ed., *14,* No. 3: 285. 1942.

This micromethod for the determination of salt in butter was developed especially for the analysis of small portions of a butter sample. The method was found to be especially useful in studying the effect of salt distribution on bacterial action in butter. An approximately 0.2-mg. portion of butter was carefully weighed, ashed and titrated with silver nitrate to determine the silver chloride present. A reasonable degree of accuracy was attained in determining sodium chloride in small samples by this method but the salt content of a microsample was not representative of a whole churning of butter. Use of the method for the study of non-uniformity in other products is suggested. For homogeneous materials it is a rapid and a precise method for chloride analysis. B.H.W.

401. **Churns and Churning—Old Facts and New Changes.** F. H. Abbott, Univ. Calif., Davis. Canad. Dairy and Ice Cream Jour., *21,* No. 3: 32. 1942.

Fat globules are sphere-shaped because of the force of surface tension. The factors tending to keep fat separated are: (1) viscosity of the liquid emulsion, (2) adsorbed material on the surface of the globule, and (3) the negative electric charge. To overcome these three forces some other force must be applied. In churning, this force is agitation, which causes collision of the globules, resulting in rupture of the material adsorbed on their surfaces. The new information regarding the process of churning created a need for changes in churn design. The older types of churns were lacking in sanitary features. Difficulty was especially experienced when they were used in making sweet cream butter. The removal of roller workers from churns has led to improved sanitation. A trend in types is toward churns that work the butter from end to end. The greatest advantage claimed for metal churns is their sanitary features. O.F.G.

402. **Dairy Developments in the Prairie Provinces.** Percy Reed, Dairy Commissioner, Regina, Sask. Canad. Dairy and Ice Cream Jour., *20,* No. 12: 19. 1941.

Production of butter in the three Canadian prairie provinces of Alberta, Manitoba and Saskatchewan has increased enormously in the last few years. These 3 provinces will produce more than 100 million lbs. of creamery butter and about 50 million lbs. of farm-made butter in 1941. The industry really became established during the terrible drought years between 1930 and 1939. Producers of butter must overcome two decided handicaps in competing for trade in eastern markets: first, that of storage with the consequent additional carrying charges, and secondly, transportation costs. The lack of wheat markets has been a factor in increased dairy production in these provinces. A continued increase is predicted. O.F.G.

403. **Studies on Surface Taint Butter. III. Some Further Characteristics of *Pseudomonas putrefaciens.*** H. Wolochow, H. R. Thornton, Univ. Alberta, and E. G. Hood, Domin. Dept. Agr., Ottawa. Sci. Agr., *22,* No. 7: 438. Mar., 1942.

Cultural characteristics of *Pseudomonas putrefaciens* are described. The strain investigated grew at 2° C. and 32° C. but not at 37° C. It did not survive 54.4° C. (130° F.) for ten minutes. Pleomorphism was displayed by cultures in LiCl and NaCl broths and the colony shape was altered on nutrient agar containing gelatin. It was always gram negative and pigment production was limited to brownish white by reflected light.

It did not show lipolysis but was markedly proteolytic when grown in skimmilk cultures.

Hydrogen ion concentrations below pH 5.48 and above about 9.5 prevented growth but this varied with the medium. Eh values were quickly and markedly reduced in glucose broth.

The action of this strain upon various sources of nitrogen and sulphur was also studied. Forty-one strains were tested in carbohydrate broths.

It had been shown previously that this organism can produce acetic, butyric and isovaleric acids from skimmilk cultures. O.R.I.

404. Developments in the Pasteurization of Churning Cream. E. L. Jack, Dairy Indus. Div., Univ. Calif., Davis. Jour. Milk Technol., *5*, No. 1: 44. 1942.

Enzymes are probably the most important agents in the spoilage of butter, and our pasteurization standards should be based upon the treatment necessary to inactivate the enzymes which may be present in the cream. The inactivation of some enzymes requires temperatures of 175 to 180° F. This is higher than that required for bacterial destruction.

In a discussion of some of the methods used for pasteurization the relative merits or disadvantages of each are given.

In the steam injection systems, subjected to vacuumization, it is possible to secure high temperatures for a short period of time, so that enzymes are inactivated, and at the same time there need be no excessive cooked flavor. To prevent this it is essential to protect the cream from direct contact with the steam jets by means of baffles. Partial homogenization, which is another detriment to be guarded against in this system, can be avoided by using a two- or three-stage vacuum treatment.

For definite inactivation of enzymes a minimum temperature of 185° F. for instantaneous heating, or 165° F. for 30 minutes should be attained, although it is possible that this temperature may be low for some of the more resistant enzymes. L.H.B.

CHEESE

405. Starter—in Relation to Cheddar Cheese Yield. E. G. Hood and C. A. Gibson, Dept. Agr., Ottawa. Canad. Dairy and Ice Cream Jour., *21*, No. 3: 24. 1942.

Lack of agreement appears to exist in the literature as to the role of starter in relation to the yield of cheese. In controlled experiments when cheese was made with 3% starter in contrast to natural ripened milk, it was found that the starter was incorporated in the cheese when yield was determined on the basis of weight adjusted to the same moisture content. The losses of protein in whey were no greater in comparative vats made with 3% starter and from natural ripened milk. No significant differences were found in the quality of the cheese made in the two ways. O.F.G.

406. A Review of Tests of Interest to the Cheesemaker. O. R. Irvine, Ontario Agr. Col., Guelph, Ontario. Canad. Dairy and Ice Cream Jour., *20,* No. 9: 25. 1941.

The premiums now being allowed for quality are an inducement to employ every possible means to ensure high quality cheese. The color developed in the resazurin test may be interpreted in terms of bacteriological quality of milk as follows: blue grey, good; lavender shades, fair; pink, poor; white, very poor. The Stormy fermentation test is designed to detect milk containing spore-forming bacteria. The B-C-P rennet test is designed to detect abnormal pH and milk which fails to clot. This test is of value only in testing milk from individual cows. The Gould rapid moisture test for cheese is described. O.F.G.

CHEMISTRY

407. Pyrolysis of Lactic Acid Derivatives. Conversion of Methyl a-Acetoxyproprionate to Methyl Acrylate. Lee T. Smith, C. H. Fisher, W. P. Ratchford, and M. L. Fein, Eastern Regional Res. Lab., U. S. Dept. Agr., Philadelphia, Pa. Ind. and Eng. Chem., Ind. Ed., *34,* No. 4: 473. 1942.

The lactose in whey and other carbohydrates may be converted into lactic acid and thence to acrylic esters and finally to acrylate resins. Methyl a-acetoxypropionate may be converted by pyrolysis to methyl acrylate and acetic acid. The effect of temperature, contact time, and various contact materials was determined. It was possible to convert virtually all of the methyl acetoxypropionate into methyl acrylate and acetic acid at temperatures above 550° C. B.H.W.

408. Ethyl Alcohol from Fermentation of Lactose in Whey. H. H. Browne, Div. Dairy Res. Labs., Bur. Dairy Indus., U. S. Dept. Agr., Washington. Amer. Chem. Soc. Jour., News Ed., *19,* No. 22: 1271. 1941.

Data are presented on the fermentation of lactose in whey by 4 different yeasts obtained from the American Type Culture Collection and by 4

Kefir yeasts isolated in the author's laboratory. A considerable difference was noted in the fermentation rates of these yeasts under the test conditions used. The highest yield, approximately 80% of theoretical, was produced by *Torula cremoris*. The mash was prepared by adjusting cheese whey to pH 4.5 with sulfuric acid, boiling and filtering it. The yeast culture together with 0.013% $(NH_4)_2SO_4$ was added. After a fermentation period of approximately 24 hours the mash was distilled until about 25% of the original volume had come over. This distillate contained approximately 14% alcohol by volume and was redistilled. The economic practicability of the process is discussed. B.H.W.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

409. New Fibre, Made from Cow's Milk, Used in Textile Manufacturing.

ANONYMOUS. Jour. Milk Technol., *4*, No. 6: 343. Nov.–Dec., 1941.

A description is given of the manufacturing process for making "Aralac" as employed by the National Dairy Products Corporation at Taftville, Connecticut. The plant has a capacity of about 5,000,000 pounds a year.

Most of the fur-felt and wool-felt hats in the U.S., Canada and South America are today being made in part with cow's milk. L.H.B.

DISEASE

410. Mastitis and the Plate Count of Milk. 1. A Quantitative Study of the Growth of Streptococcus Agalactiae in Various Plating Media.

MAX E. MORGAN, E. O. ANDERSON, AND W. N. PLASTRIDGE, Depts. of Dairy Indus. and Animal Diseases, Univ. Conn., Storrs, Conn. Jour. Milk Technol., *4*, No. 5: 245. Sept.–Oct., 1941.

In order to determine the growth promoting properties of various media for *Str. agalactiae*, a series of 20 freshly isolated cultures of the organism were grown in milk and then were plated in four different media, namely: (1) 5% oxblood agar, (2) Edwards' crystal violet aesculin oxblood agar, (3) the new standard medium and (4) the old standard medium. It was found that the blood agars supported more colonies than did the agars containing no blood. There was no significant difference in the growth-promoting ability of the plain oxblood medium and Edwards' medium. Neither was there any significant difference in this respect between the new and the old standard media. However, the mean exposed area of the colonies on the new standard medium was 420.3% larger than the mean exposed area of the colonies on the old standard medium. Thus, on the new standard medium the colonies of *Str. agalactiae* were large enough so that they would not be classified as "pinpoint" colonies. L.H.B.

411. A Discussion of Mastitis. H. L. Davis, W. H. Kimmer, and J. A. Anderson, Filter Products Div., Johnson and Johnson, New Brunswick, N. J. Jour. Milk Technol., *5,* No. 1: 18. Jan.–Feb., 1942.

A "footnote" concerning this paper as published gives an abstract of its contents and was as follows: "This paper attempts a constructive survey and review of some recent work, and presents concepts of the cause, cure, and prevention of mastitis. It is presented as a service to the dairy industry, whose cooperation with veterinarians will hold this disease under control."

A number of tests for detecting mastitis are discussed, and it is concluded that although each of them has certain advantages, none can be relied on to give a positive answer on only one sample of milk; it is necessary to use repeated testing.

It is concluded that mastitis is a preventable disease, and that recent tests indicate that it may soon be classed as curable. L.H.B.

412. Report of the Committee on Communicable Diseases Affecting Man. Internatl. Assoc. Milk Sanit., Horatio N. Parker, Chm. Jour. Milk Technol., *4,* No. 4: 223. July–Aug., 1941.

There were 42 disease outbreaks in 1938 in the U. S. Eighty per cent of the outbreaks were in communities of less than 5,000, and only one in a community of 100,000.

Only one outbreak was attributed to pasteurized milk, and in this case it was reported that the pasteurizer had broken down. L.H.B.

413. The Control of Mastitis. L. E. Bober, Babson Bros. Co., Chicago, Ill. Jour. Milk Technol., *4,* No. 3: 152. May–June, 1941.

A program for herd management to combat mastitis is given.

Instances are given in which such a program has been very beneficial in controlling the disease.

The fundamental rules suggested are:

1. Detection of infected cows by chemical, physical and bacteriological tests. 2. Segregation, isolation or sale of infected cows, depending on economic factors. 3. Prevention of injury, strict hygienic care in milking and barn conditions, and a feeding program to foster the highest degree of health and resistance. 4. Testing all new additions to the milking herd before placing them in the herd. L.H.B.

414. A Milk-borne Typhoid Outbreak Traced to Dairy Water Supply. T. R. Meyer, Rosemary Phillips, H. E. Lind, and Leonard M. Board, Health Comn., Dirs. of Nursing, Lab., and Sanit. Divs., St. Louis County (Mo.) Health Dept., Clayton, Mo. Jour. Milk Technol., *4,* No. 3: 123. May–June, 1941.

The dairy involved had previously produced milk for a St. Louis plant, but because of failure to comply with the provisons of the City Health Dept. had had its permit revoked about two years previous to the outbreak. The sale of raw milk resulted at the farm, with customers furnishing their own containers which were frequently rinsed with water at the milk house.

The typhoid fever outbreak resulted in 26 cases in 10 families and was traced to this small cash and carry raw milk dairy. The dairy water supply (a cistern) was established as the source of infection. Samples of the water contained *B. typhosus* organisms. L.H.B.

415. Diseases and Ailments of Dairy Cattle with Which a Milk Inspector Should be Acquainted. ANDREW W. UREN, Vet. Dept., Univ. Mo. Jour. Milk Technol., *5,* No. 1: 48. Jan.–Feb., 1942.

A brief discussion is given of the various diseases which may be transmitted from the cow through the milk. Examples are cited in some instances. L.H.B.

FEEDS AND FEEDING

416. Preservation of Grass Silage by New Methods. W. E. KRAUSS, Ohio Agr. Expt. Sta., Wooster. Certified Milk, *16,* No. 183: 5. July, 1941.

Molasses or phosphoric acid is now generally used in making grass silage. A preservative of this type involves additional expense and some inconvenience. The new methods suggested are: (1) the use of a preservative usually available on the farm, and (2) using no preservative at all, but increasing the dry matter content of the silage. An example of the first method is cheese-factory whey, which has been used with some success in developing a desirable fermentation. The possibilities of the second method were demonstrated with more than fifty silo fillings, which showed that when the dry matter content was right, the untreated material was equal to that of the preserved silage. The satisfactory range of dry matter, between which good silage can be made without a preservative and without undue loss of juice, was found to be 25 to 40%. The dry matter content of the green material can be increased, by introducing into the cutter, along with the green feed, such materials as dry hay, stover, straw, ground corn or oats. W.S.M.

417. The Effect of Shark-liver Oil on the Vitamin A Content of Milk and on Milk Production. H. J. DEUEL, JR., Univ. So. Calif., Los Angeles, Calif., AND J. P. NUTTALL, Los Angeles Medical Milk Comn. Certified Milk, *16,* No. 186: 3. Oct., 1941.

The effect of the addition of shark-liver oil to the diet of Guernsey cows was studied both from the standpoint of the vitamin A content of the milk and from that of total milk and butter fat production. It was found that the administration of shark-liver oil in quantities of 700,000 I.U. or over per cow per day increased vitamin A in the butterfat. A value as high as 170 units per gram was found in one case. The increase of vitamin potency was largely in the form of vitamin A rather than as carotene, and the increase continued for 23 weeks during which the supplement was fed. The shark-liver oil also increased milk production and possessed no toxic properties similar to that of cod-liver oil, which results in a lowering of percentage of butter fat. A suppression in carotene excretion was also noted. W.S.M.

FOOD VALUE AND DAIRY PRODUCTS

418. More Education Is the Real Answer to More Milk in the Diet. E. M. HARMON, Natl. Dairy Council, Chicago, Ill. Canad. Dairy and Ice Cream Jour., *21,* No. 1: 21. 1942.

In the great middle classes where most of the market is, the percentage of income spent for food is nearly the same in each case. It is possible to conclude, however, that purchasing power is largely the answer to the problem of getting more milk into the diet. The matter of price, however, has been over-played, especially by economists and social workers. With all credit to the sincere efforts put forth by governmental agencies to solve the problem of low-cost milk, the results of those very efforts raise serious questions as to their value when considered alone in solving the malnutrition problem in low-income areas. Consumer education in the lower income groups is more necessary because of the fact that with limited amounts of money to spend they simply cannot afford to make mistakes in the selection of food. Observations over a period of years in communities where consumer education programs are operating are another evidence of the necessity of consumer education. In consumer education it should be stressed that milk is the nutritional foundation of an adequate diet, that it is palatable and has a bland flavor, and that it is an economical food. O.F.G.

419. A Study of the Food Value of Various Dairy Products. H. H. MITCHELL, Univ. Ill., Urbana. Canad. Dairy and Ice Cream Jour., *20,* No. 10: 104. 1941.

A good method of expressing the nutritive value of milk is to estimate what proportion of the nutritive requirements of a man would be supplied by a moderate amount of milk. Taking 3.5% raw summer milk as a standard to be compared with daily nutritive requirements of a man of average weight, the best information indicates that a quart of milk will provide approximately the following percentages of the daily needs:

Protein	73%	Vitamin A	95%
Energy	21%	Vitamin B	52%
Calcium	150%	Vitamin C	100%
Phosphorus	60%	Vitamin G	100%
Iron	3%	Nicotinic acid	28%
Copper	4%		

One quart of summer milk will provide only 7% of the quantity of vitamin D needed daily by a child. These values show that cow's milk is a very poor provider of iron, copper and vitamin D, only a moderately good provider of energy, nicotinic acid and vitamin B_1, a somewhat better provider of phosphorus and protein, a very good source of vitamin G, vitamin C (under the most favorable conditions), and vitamin A, and an excellent source of calcium. The evaporation and drying of milk may cause variable and generally slight destruction of the less stable constituents of milk. Vitamins A and G are quite stable to processing methods. Preparation of choice dried milk may lower the nutritive value of milk proteins about 8%. Criticism is offered to the practice of correlating animal preference for a food to the nutritional value of that food. Lactose exerts a much more favorable effect on calcium utilization than cane sugar or glucose. Orange juice, and possibly other fruit juices, have been shown to improve the utilization of milk calcium in the animal body. The addition of certain fruits and their juices to ice cream increases the vitamin C content of this product. The author discusses recent researches on the effect of the ingestion of very cold foods and drinks on the stomach and the heart and on the cooling mechanism of the body. O.F.G.

420. Nutritional Value of Milk and Pasteurization Process. C. A. Elvehjem, Univ. Wis., Madison. Canad. Dairy and Ice Cream Jour., *20,* No. 10: 95. 1941.

Milk is deficient in copper, iron and manganese. The addition of the first two to milk prevents the development of anemia which is characteristic of unsupplemented milk diets. The difference in animal growth in summer- and winter-produced milk is attributed to the grass juice factor content of the two milks. The conclusion has been reached that the kind of milk used for pasteurization is more important than changes which may occur during the process of pasteurization. Results indicate slight deficiencies of vitamin E, and perhaps vitamin K, when animals are continued on heat-treated milk through reproduction. Pasteurization does not lower the vitamin A and carotene content of milk although prolonged boiling may destroy as much as one half of the original vitamin. The only two nutritional factors which may be reduced in milk during pasteurization are vitamins C and B_1. With present practices of adding vitamin B_1 to flour the slight loss occurring in pasteurization is not significant. O.F.G.

ICE CREAM

421. The Need of Sanitary Control in the Dispensing of Frozen Dairy Products. F. W. FABIAN, Res. Prof. Bact., Mich. State Col., East Lansing, Mich. Jour. Milk Technol., *4,* No. 5: 285. Sept.–Oct., 1941.

Observations indicate that frequently sanitary precautions required in the production of frozen desserts are often nullified by improper dispensing methods, untrained personnel, and inadequate facilities for properly washing and sterilizing dishes and utensils used. L.H.B.

422. Continuous Freezer in Making Fancy Ice Cream. G. O. WYMOND, Cherry-Burrell Corp., Chicago, Ill. Canad. Dairy and Ice Cream Jour., *20,* No. 9: 56. 1941.

A knowledge of certain fundamentals is necessary for the successful making of fancy ice cream molds. The design of the center mold should not be too intricate or attempt to show too much detail. The ice cream going into the center mold must be very stiff and that forming the body around the center somewhat softer. The center core should move at the same rate of speed as that of the cream forming the body. The continuous freezer adapts itself naturally to the making of "wave" ice cream. Directions are given for using the continuous freezer in the making of fancy designs such as cakes and pies. O.F.G.

423. Sandiness in Ice Cream—Its Causes and Control. H. H. SOMMER, Univ. Wis., Madison. Canad. Dairy and Ice Cream Jour., *20,* No. 9: 23. 1941.

Sandiness became a problem in ice cream manufacture when the industry started to build up the serum solids content. Sandiness is actually due to the formation of lactose crystals and this involves lactose solubility in water. According to Dahle there should be 6.4 lbs of water for each lb. of serum solids if sandiness is to be avoided. Under some conditions the ratio may be as low as 1 to 5.9. The following formula may be used for calculating the upper limit of serum solids to be used in a mix:

$$\frac{100-X}{6.9} = \% \text{ serum solids (upper limit)}$$

where X stands for all the solids other than the serum solids. Sandiness seldom develops in the hardening room at 0° to −10° F. This condition does not hold in retail cabinets. Tests showed that sandiness developed more rapidly at 12° F. than at lower temperatures. The author offers an explanation for the failure of sandiness to develop in ordinary hardening-room practice. As freezing takes place less and less of the water is in the liquid state and the lactose solution becomes more concentrated. Finally a state is reached in which the sugar solution is so viscous that lactose crystallization cannot take place. A glass state is formed. If the temperature remains for a sufficient length of time in a range favorable to crystallization and the solution is not viscous then sandiness will develop. Even at favorable temperatures this usually requires 5 to 7 days. The addition of calcium and magnesium salts before homogenization delays sandiness. Clarification of mix ingredients and preliminary soaking of added nuts in sugar solution are helpful practices. O.F.G.

424. War-Time Ice Cream in England. CLIFFORD SKERTCHLY, "The Ice Cream Industry," London. Ice Cream Trade Jour., *38*, No. 2: 12. 1942.

During the last season England produced 25,000,000 gallons of ice cream, or approximately 50% of the normal output. This was done in spite of an almost complete lack of butterfat, serum solids, and eggs, and a shortage of sugar and flavors. The United States will probably never face such severe shortages, but may nevertheless profit from the experiences of the English. The English have been using margarine fats to replace butterfat, and wheat and potato flour to replace the serum solids. The flour should be used in amounts not to exceed 50% of the skim milk powder. The use of wheat flour with condensed milk is not mentioned, and apparently condensed milk is not available to English manufacturers.

Eggs greatly improve mixes made with flour and some eggs imported from the U. S. are available. The English public has been very tolerant of the use of substitutes in ice cream and in one instance a manufacturer sold "vanilla" ice cream for three days which had no flavoring at all in it. W.H.M.

425. Ice Cream in the Last War. VINCENT M. RABUFFO, Ed. Ice Cream Trade Jour., *38*, No. 2: 8. 1942.

A chronological history of the first World War reveals that the frozen desserts manufacturer faced about the same problems then as he does now. A shortage of essential equipment, supplies and labor had to be met. Dairy products purchased by Europe were 456% as great in 1915 as 1914 so that the problem of increased demands was also felt. Food administrator Herbert Hoover classified the ice cream business as an essential industry and this helped the manufacturer in obtaining equipment and supplies. Sherbets and ices, however, were banned. Leaders of the industry recommended greater all-around efficiency as a means of combating the extra costs. The great advantage of the first war to the ice cream manufacturer was the increased skill in salesmanship and advertising. W.H.M.

426. Honey as a Sweetening Agent. Paul H. Tracy, Prof. Dairy Mfrs., Univ. Ill., Urbana. Ice Cream Trade Jour., *38*, No. 3: 21. 1942.

Honey contains 80% solids and is 75% as sweet as sucrose. If a distinct honey-flavored product is desired, 9% honey and 7% sucrose should be used. If no honey flavor is desired, no more than 2% honey can be used. Honey blends well with fruits, but not vanilla and is covered up by chocolate. Honey ice cream does not whip as readily, will be softer in the dealer's cabinet and will melt more readily. These are not serious objections. The texture is better, but the body of the honey ice cream may be slightly crumbly or sticky. There are various flavors of honey and the mild flavors are best. Sweet clover, alfalfa, basswood, orange and tupelo are particularly desirable. Honey can be used as toppings for sundaes and in making ripple ice cream. The lowered freezing point of honey ice cream may be overcome by lengthening freezing time slightly and lowering storage and cabinet temperatures. W.H.M.

427. The Wage-Hour Law in the Ice Cream Trade. Colin Kerr Cameron, Wage and Hour Div., U. S. Dept. Labor. Ice Cream Trade Jour., *38*, No. 2: 16. 1942.

The average neighborhood ice cream store is exempt from the provisions of the Wage-Hour Law. Manufacturers engaged in interstate commerce and also engaged in retail business not in interstate commerce may claim exemption for those retail employees under specified conditions. Employees of a local plant receiving goods which have been shipped across a state line are covered by the law. Executives, salesmen, and administrative officers are in most cases exempt. Men employed within the area of production (as defined by the government) and making ice cream or processing other dairy products are exempt from the law. Manufacturers are not required to count as "hours worked" any time the employee is on the premises and not working due to blackouts or air raid alarms. Manufacturers and retailers are advised to check the legal phrasing of each particular instance when there is a question as to whether an employee is covered by the law. W.H.M.

428. Retail Prices? Vincent M. Rabuffo, Ed. Ice Cream Trade Jour., *38*, No. 3: 10. 1942.

Retail prices on ice cream and items sold at soda fountains have been revised upward all over the country. The manufacturer's problem is to keep the retail price down to where it will maintain sales volume. This can be partially accomplished by dealer education. Various methods have been used to maintain a margin above cost of ingredients. Dealers in the East have advanced five cent cones and novelties to six or seven cents and ten cent sodas to twelve or fifteen cents. In other sections the five cent price has been retained, but double dips are no longer used and dipper sizes have been cut. Ten cent sodas have been raised to twelve cents or fifteen cents with an increase in ingredients. Sundaes are up five cents generally. Where prices have gone up five cents, most generally the quantity of ice cream has been raised. Where prices are the same the quantity has been cut, and where prices have advanced two or three cents, the quantity remains the same. W.H.M.

429. Ice Cream Delivery in Wartime. ROBERT T. SMITH, Ice Cream Consultant, Scranton, Pa. Ice Cream Trade Jour., *38*, No. 4: 10. 1942.

A survey in the East reveals that 82½% of the ice cream dealers have reduced general delivery schedules. These reductions varied from 10% to 50% with the average reduction of 25 to 30%. Forty-five per cent are eliminating certain dealers so that they may use new plans of distribution and 67½% are installing additional cabinets for reserve stocks to lessen deliveries. In changing schedules, a manufacturer should consider an analysis of dealers, a study of routes, the number of flavors handled, effect of new schedules, and his competitive relation. It is not known when more rubber will be available, but a manufacturer should figure that his present supply must last until 1944. The elimination of small troublesome dealers, special deliveries and long routes may actually increase profit as well as save rubber. W.H.M.

430. Factors Affecting the Body of Ice Cream. B. I. MASUROVSKY, Res. Ed. Ice Cream Trade Jour., *38*, No. 4: 38. 1942.

The body of ice cream is the net result of the individual arrangement of small particles, this arrangement being referred to as the texture. There are two factors affecting the body, namely, physico-chemical makeup and mechanical treatment. Under the first are included acidity, viscosity, surface tension, overrun, emulsification of fats, suspension of milk-solids-not-fat, enzymes, stabilizing agents, colloidal protection and others. Mechanical factors are homogenization, temperature control, incorporation of air cells and freezing and hardening of the finished ice cream. Commercial plants should stay within the limits of 90 to 100% overrun, to avoid sogginess or the other extreme, fluffiness. A good body and texture are no longer hard to obtain under practical conditions. W.H.M.

431. Conserving Chocolate Coating. J. HOFFMAN ERB, Dept. Dairy Technol., Ohio State Univ., Columbus. Ice Cream Trade Jour., *38*, No. 4: 28. 1942.

Due to the lack of cocoanut oil, manufacturers cannot obtain quantities of "pail" coating and will have to use pure chocolate slab coating for ice cream bars. It is more difficult to maintain the proper viscosity of slab coating due to water pickup causing progressive thickening. Moisture should be kept out of the coating as much as possible and the addition of 0.2% to 0.3% lecithin helps to overcome the effects of any water which does get in. Most coatings already contain lecithin. The manufacturer should standardize the fat content of the coating at 55 to 60%, and he should purchase a properly milled product. Temperature of the coating and ice cream should be proper and uniform. Coating containing milk solids should not be heated over 115° F. The dipping temperature of slab coating is higher than pail coating, ranging from 105 to 115° F. A small metal bob may be cooled, weighed, dipped and reweighed to determine the coverage value of a coating. The bob would approximate a small ice cream bar and conditions should be the same as actual dipping. W.H.M.

432. **Right Merchandising.** James H. Meehan, Dir. Sales and Advertising, Phila. Dairy Products Co. Ice Cream Trade Jour., *38*, No. 2: 38. 1942.

The Philadelphia Dairy Products Company, Inc., has provided a merchandising course for their representatives. The course covers the handling of their product in the retail store and plant. In addition, it provides information about window displays, cleanliness, store arrangement, cost analysis, lighting, courtesy and other helpful material to be used by the representatives and passed on to the store owner. The school works on the theory that business should come from an increased sales volume from present retail outlets and not from competition to secure other companies' outlets. Also the way to improve sales volume through their stores is to help make the entire store a better arranged, better advertised, cleaner, and more profitable business. W.H.M.

MILK

433. **A Comparison of Dowicide-A and Chlorine (Diversol) for Use in Milking Machines.** F. W. Fabian and G. L. Nielson, Bact. Sec., Mich. Agr. Expt. Sta., East Lansing. Jour. Milk Technol., *4*, No. 5: 268. Sept.–Oct., 1941.

In comparative tests as a bactericide for milking machines it was found that a solution of 1:200 Dowicide-A compared favorably with a chlorine solution containing 102–188 p.p.m. of chlorine.

It was found to be even more stable than the chlorine solution, being usable for a week and even longer without an apparent loss of germicidal efficiency.

Results also indicated that it was much faster acting than the chlorine solution under the conditions of the experiments.

Under the conditions of the experiment the Dowicide-A (sodium ortho-phenyl-phenate) did not have any effect on the phosphatase test of the milk. It was found that it was necessary to have Dowicide-A present in a concentration greater than 1:50,000 before a positive test was obtained.

L.H.B.

434. Influence of Processing on the Properties and Flavor of Milk. P. F. Sharp, Cornell Univ., Ithaca, N. Y. Certified Milk, *16*, No. 184: 9. August, 1941.

The chief objectives in processing milk are: (1) reduction of the sanitary hazard to the vanishing point, (2) prevention of the entrance of and control of the growth of bacteria, (3) prevention of non-bacterial chemical changes and control of physical properties. Faulty processing may result in (1) milk-borne epidemics, (2) development of undesirable flavors in milk, (3) loss in nutritive value, and (4) undesirable alterations in physical properties. Discussed factors which affect flavor, nutritional value and physical properties are: (1) control of bacterial content and growth, (2) lipase, (3) creaming, (4) oxidized flavor, (5) vitamins C, A, G, D, and B_1, and (6) deaerated milk. The author suggests that efforts should be continued to produce better milk from the standpoint of nutrition and flavor. W.S.M.

435. High-temperature, Short-time Pasteurization and Its Practical Application to the Dairy Industry. J. L. Hileman and Henry Leber, Dairymen's League Cooperative Assoc., Inc., Syracuse, N. Y. Jour. Milk Technol., *4*, No. 3: 128. May–June, 1941. (Also published in the Annual Proceedings, N. Y. State Assn. of Dairy and Milk Inspectors, 1941.)

Advantages of the high-temperature, short-time method were given as follows:

1. Economy of floor space. 2. Elimination of some of the personal element by the use of automatic controls. 3. Elimination of over-holding of milk. 4. Elimination of the thermophilic problem. 5. Elimination of certain chances of contamination of the product because the entire apparatus is a closed system that cannot be opened during operation. 6. Economy in the use of heat and refrigeration. 7. Small temperature differential between heating medium and milk, with less danger of cooked flavors and other undesirable effects of overheating.

Disadvantages listed were:

1. Lack of flexibility, especially in large installations. 2. Smaller margin of safety due to extremely short holding time. 3. Higher bacterial

counts with many milk supplies, with greater increased cost of laboratory and field work to hold these higher counts down. 4. Not only are total bacterial counts higher, but in at least some milk supplies, the percentage of alkali-producing bacteria is higher. Several studies made on both commercial and laboratory pasteurization are reported.

No significant difference was found in cream volume per one per cent of fat when milk was pasteurized commercially at 143° F. for 30 minutes and 161° F. for 16 seconds. The average creaming factor or per cent of cream for 1% of fat for milk pasteurized at 143° F. for 30 minutes was 3.45 and for milk pasteurized at 161° F. for 16 seconds was 3.52.

The phosphatase test was reported satisfactory on all samples of commercially pasteurized milk by this process (161° F.—16 sec.) and the colon count was satisfactory on 94.5% of the samples studied.

Samples of milk pasteurized commercially at 161° F. for 16 seconds were also pasteurized in the laboratory at 143° F. for 35 minutes and at 161° F. for 16 seconds. Where the bacterial counts on the milk pasteurized in the laboratory at 161° F. for 16 seconds were low, there was close agreement between the two laboratory methods, but they differed more and more as the counts became higher, and 161° F. for 16 seconds gave the higher counts. Where the counts on samples pasteurized in the laboratory at 161° F. for 16 seconds were below 5,000, the commercially pasteurized samples were nearly five times higher, but the difference became less as the counts increased.

Inspection of farms where thermoduric counts were high usually disclosed the cause to be dirty milking machines, pails or strainer.

The use of weak acids for cleaning the stainless steel plate heaters was found to be more satisfactory than alkaline cleansers. The procedure used was to flush the system with cold water, then circulate dilute phosphoric acid (0.1%) through the system at 160° F. for 30 to 60 minutes. Flush with cold water and then circulate a mild alkaline solution at 160° F. for 30 to 60 minutes. (Solution may contain one to two pounds of soda ash, tri-sodium phosphate or sodium silicate per 90 gal. of water.)

A special pump should be provided for the cleaning operation to prevent damage to the milk pump from the cleaning agents.

Plates should then be opened and brushed thoroughly and rinsed free of alkali. L.H.B.

436. Aspects of Public Health Legal Control of Paper Milk Containers.

C. O. Ball, Amer. Can Co., N. Y. Jour. Milk Technol., *5*, No. 1: 6. Jan.–Feb., 1942.

This discussion includes a brief review of findings of various investigators and a discussion of some of the findings, including the number of organisms in paper used for making containers; various apparatus used for disintegrating paper for bacteriological examination; specifications of temperature used for paraffin treatment, and methods for determining the sterility of containers.

The author summarizes as follows:

Apparatus required for the bacteriological examination of paper is comparatively simple and inexpensive.

"The number of organisms in paper used in containers is of little public health significance, and the relationship between paper mill sanitation and the number of bacteria in finished paper has not been definitely established."

Because of inherent inaccuracies in laboratory methods of determining bacteria counts and the fact that they are not sufficiently precise, they do not warrant the stressing of individual counts; logarithmic average is probably the fairest method of dealing with such data.

It has been found by investigators that paraffining paper milk containers at 165° to 170° F. was better than paraffining them at 180° to 185° F.

Indications are that when interpreting the results from the testing of containers for sterility by the rinse technique that laboratory contamination is responsible for a large proportion of positive findings. L.H.B.

437. **A Small Electric Holder Type Pasteurizer.** C. W. ENGLAND, ARTHUR P. WIEDEMER, AND GEORGE J. BURKHARDT, Md. Agr. Expt. Sta., College Park, Md. Jour. Milk Technol., *4*, No. 4: 187. July–Aug., 1941.

A description and specifications for a small electrically operated pasteurizing unit, of the holding or batch type, having a capacity of twelve gallons of milk is given.

After several months of operation, the authors found the pasteurizer to be safe, efficient and practical, and its operating cost to be low where average electric rates are available. Using current at 2 cents per kw.hr., the cost for pasteurizing 12 gallons of milk varied from 0.33 cents to 0.61 cents per gallon depending on the temperature of the raw milk at the start of the operation. L.H.B.

438. **Sanitary Regulations for Controlling the Production of Paper Containers for Milk.** C. N. STARK, Prof. of Bact., Cornell Univ., Ithaca, N. Y. Jour. Milk Technol., *4*, No. 4: 200. July–Aug., 1941.

The author presents the idea that lower cost of milk to the consumer would have a tendency to increase milk consumption particularly by the lower income families. That the paper milk bottle appears to be a means of obtaining a lower price is suggested. It is stated that "any unwise or unnecessary regulations in the production of paper containers for milk will eventually contribute toward an increased cost to the consumer, and a decreased consumption of milk, and a generally lowered health condition of the people." L.H.B.

439. Physical Structure of Milk. E. L. Jack, Univ. Calif., Davis. Canad. Dairy and Ice Cream Jour., *21*, No. 3: 42. 1942.

Milk is a complex material composed of a number of constituents existing as individual particles. The size, shape and inter-relationship of these particles comprise the physical structure of milk. Milk is a liquid, not because it contains 87.25% water, but because of the size and shape of the particles composing it. The volume percentage of space occupied by the solids of milk is about 10.30, the water 89.70. The fat globules are the largest particles in milk. The particles of the separate materials are essentially globular resulting in a fluid. The protein particles may have their shape altered through the action of acids or other agents. The sugar and protein particles are heavier than water but are prevented from settling out because of viscosity and the electrical forces that are effective on particles as small as these. The fat particles rise but are prevented from running together because of the material adsorbed on their surfaces. O.F.G.

440. Bacteriological Problems of Pasteurization. W. C. Frazier, Univ. Wis., Madison. Canad. Dairy and Ice Cream Jour., *21*, No. 1: 19. 1942.

With the increasing use of "high-short" methods of pasteurization, more information is needed on the survival of bacteria and changes in the products due to heat. The purpose of pasteurization is to destroy microorganisms and enzymes and leave the product as little changed as possible. The resistance of a single kind of bacterium to pasteurization will vary, depending upon certain factors. Clumping increases resistance and the older organisms are more resistant than young ones. The presence of large numbers of thermoduric bacteria in milk is significant because it will mean a high percentage of survival after heating. Thermophilic bacteria increase in number in milk kept at above 100° F. Bacteria of the Colon Aerogenes group usually are killed by accepted methods of pasteurization. The presence of sugar, gelatin and dried evaporated milk in ice cream protects bacteria against heat to a certain extent. The preparation of starters for fermented milks and for cheese involves the use of milk heated differently. Lactic bacteria for butter, cultured buttermilk or cheese can be grown in milk pasteurized at 180–200° F. for 30 minutes.

The proteases, enzymes which break down proteins, are fairly resistant to heat. Lipase, the fat-splitting enzyme, is destroyed by pasteurization. Peroxidase is destroyed by heat treatments a little greater than the ones employed for market milks. Phosphatase is destroyed by efficient pasteurization. O.F.G.

441. Bacteriological Principles of Pasteurization. E. G. Hastings, Univ. Wis., Madison. Canad. Dairy and Ice Cream Jour., *21,* No. 1: 17. 1942.

This article presents the history and development of heat treatment as a means of preserving and making food safe for human consumption. The author concludes that the advantages of pasteurization far outweigh its disadvantages and states that it is one of the chief factors in increasing the wider use of milk. O.F.G.

442. Creamery Problems in a War-time Economy. C. E. Lackner. Canad. Dairy and Ice Cream Jour., *20,* No. 12: 44. 1941.

The shortage of male labor is increasing; consequently, plans should be made for training female labor for replacement. Cream cans must receive extreme care since they are almost impossible to replace because of a shortage of metals. Improvement in the methods of washing and caring for cream cans is needed. The gasoline shortage demands that overlapping of truck transportation be eliminated or lessened. The quality of creamery butter is holding up well considering the war conditions. O.F.G.

443. Some Aspects of the Canadian Dairy Industry in 1941. J. F. Singleton, Assoc. Dir. Marketing Service, Dairy Products, Dept. Agr., and Chairman, Dairy Products Board. Canad. Dairy and Ice Cream Jour., *20,* No. 11: 36. 1941.

This article discusses the growth of the Canadian dairy industry during the past 75 years and shows how, during the first half of that period, the increases in the production of dairy products greatly exceeded the increase in domestic consumer demand. Recently there has been a shortage of cheese and particularly of relatively fresh cheese for home consumption because of the amount being exported to Britain. The amount of butter in storage during 1941 was greater than at the close of 1940. The anticipated increase in domestic requirements for concentrated milk has been realized and distribution of evaporated milk during the first 8 months of 1941 shows an increase of about 30%, and distribution of sweetened condensed milk shows an even greater percentage of increase. Reports indicate a decided increase in consumption of fluid milk and ice cream the first 9 months of 1941.
O.F.G.

444. Bacteria—Heat-resistant and Heat-loving. W. B. Sarles, Univ. Wis., Madison. Canad. Dairy and Ice Cream Jour., *20,* No. 9: 27. 1941.

This article discusses the nature and control of thermophilic and thermoduric organisms. The following steps can be taken to avoid building up thermophilic flora in a plant:

1. Cans and tanks must be clean and free from cracks and crevices. 2. Equipment surfaces must be free from milk film and milk stone. 3. Dead-ends, leaky generators and vats that drain poorly must be eliminated. 4. Use an air-space heater to make sure of destruction of bacteria in foam. 5. Do not repasteurize pasteurized or partially pasteurized milk. 6. Avoid long, continuous runs with vat pasteurizers. 7. Do not use the same filter cloth on hot milk over a long period of time. 8. Use high-temperature, short-time pasteurization if other control methods fail. Thermoduric bacteria must be kept out of milk because they cannot be removed or destroyed by pasteurization. O.F.G.

445. Pasteurization of Milk and Public Health Results. F. J. Moss, U. S. Public Health Service, Washington, D. C. Canad. Dairy and Ice Cream Jour., *20,* No. 9: 19. 1941.

An annual survey of milk-borne diseases was instituted by the Office of Milk Investigations of the Public Health Service in 1923. Within the years 1923–1938, an average of 43 outbreaks per year was reported. Typhoid fever accounted for the majority of the outbreaks, while septic sore throat and scarlet fever, with about equal percentages of the total, were the next two most prevalent kinds of outbreaks. During this period about 95% of the outbreaks and cases involved raw milk and raw milk products. A careful study showed that children who were fed pasteurized milk thrived as well as children who received raw milk. Pasteurization now has as its purpose, not the prevention of souring, but instead the prevention of disease. The conclusion drawn by this article is that all milk should be pasteurized. O.F.G.

446. High-temperature Pasteurization from the Small Operator's Viewpoint. A. C. Dahlberg, N. Y. Agr. Expt. Sta., Geneva, N. Y. Internatl. Assoc. Milk Dealers, Assoc. Bul., *34,* No. 8: 174. Jan., 1942.

For the small operator special disadvantages are: more expensive and more complicated, lacking in flexibility in handling a variety of products in small lots, time necessary for cleaning equipment relatively excessive, skilled help not always available and the equipment cannot be hand-operated. In spite of these drawbacks there is much enthusiasm for the process on the part of small dealers. Results of experimental work with higher than usual temperatures called quick-time pasteurization are given. The pasteurization time is the total time in seconds from 140° F. to the maximum temperature and back to 140° F. with no holding time at maximum temperature. It is believed that a wider temperature margin of safety is present when heating to 170° F. and back to 140° F. on 12 sec. than in conventional systems of short-time, high-temperature pasteurization, since 167½° F. gave a negative phosphatase test and 172½° F. was attained before the creaming ability was injured. Comparable pasteurizing results would be obtained by the following:

Time above 140° F.	*Highest temperature*
5 seconds	177.5° F.
6 seconds	175.0° F.
12 seconds	170.0° F.
24 seconds	169.0° F.

The flavor of the milk heated to 177.5° F. was less cooked than low-temperature pasteurized milk. High velocity of milk in the pasteurizer permitted a high heating water temperature and practically eliminated milk stone. E.F.G.

447. **Homogenized Milk—Plant View.** Ross J. Quirie, United Farmers Coop. Creamery Assoc., Boston, Mass. Internatl. Assoc. Milk Dealers, Assoc. Bul., *34,* No. 8: 188. Jan., 1942.

A holding period of 15 hours at 72° F. is recommended as a quality test. No sample should rise more than .05% in acidity. Temperatures of pasteurization as high as 170° F. for 30 minutes are reported to give good results. A pressure above 3000 lbs. per sq. in. with a piston machine seems to be needed. Returns may be best utilized in chocolated milk, buttermilk and ice cream mix. E.F.G.

448. **Homogenized Milk—Laboratory View.** D. L. Gibson, The Borden Co., Toronto, Canada. Internatl. Assoc. Milk Dealers, Assoc. Bul., *34,* No. 8: 179. Jan., 1942.

Efficiently homogenized milk after 48 hours should contain less than 5% more fat in the upper 50 ml. of the bottle than in the mixed remainder. Or leave a bottle on the laboratory desk overnight and examine for cream plug and flavor. Under the microscope all fat globules should be under two microns in diameter and preferably less than one.

Milk heated to 154° F. and homogenized at 2500 lbs. pressure will leave the homogenizer at 160° F. and this is not too high for the holding method. 170° F. for 15 seconds is good for the short-time high-temperature method. Any temperature from 120° F. up is satisfactory for homogenization which is preferably done prior to pasteurization with just enough pressure to produce good homogenization. The order should be forewarm, filter or clarify, preheat, homogenize and pasteurize.

Sedimentation is not likely to occur with less than 200,000 cells per ml. However, high storage temperature and returns favor it. Clarification is more effective than filtration although the latter is often satisfactory with high quality milk. The high pasteurizing temperature should result in a better than average keeping quality. A light cream line or cream plug may be caused by (a) running "regular" milk first without flushing pipes, (b) hot milk run into colder homogenized milk, (c) first milk through homogenizer, (d) inaccurate gauge, (e) clarification after homogenization, (f) valves need grinding.

Complaints involve: (1) serum formation due to freezing, (2) not good for junket or custard, (3) rancid off flavors, (4) cappy or cardboard flavor.

A 4.0% fat milk is ideal. Since the Babcock test yields results 0.05 to .15% low it is best to allow at least .1% above legal minimum. Grind the valves and agitate after each 300,000 gallons of milk. E.F.G.

449. A Babcock-test Reading Device. L. M. LAMPERT, State Dept. Agr., Sacramento, Calif. Jour. Milk Technol., *4*, No. 6: 318. Nov.–Dec., 1941.

A description and illustrations of a new light-weight instrument that fastens to the hand. L.H.B.

450. *Staphylococcus Aureus* Contamination of a Grade "A" Raw Milk Supply. W. L. WILLIAMS, Univ. Louisville, Louisville, Ky. Jour. Milk Technol., *4*, No. 6: 311. Nov.–Dec., 1941.

Staphylococcus organisms were found to be present in all ten of the raw market milk supplies examined. Many were of the aureus type and a few were of the white albus type. Most of the strains isolated were classified as potential pathogenic strains.

On examination of samples of aseptically drawn milk from 387 animals in the ten herds, it was found that over 50% of the animals had staphylococci in their milk.

"The realization of the seriousness of a possible milk-borne food poisoning epidemic led the raw milk producers to a decision for 100% pasteurization of the raw milk supply." L.H.B.

451. A Comparison of Microscopic and 32°–T–G–M Agar Plate Counts on Raw Milk. HERBERT JENKINS, Dir. Labs., New England Dairies, Inc., Boston, Mass. Jour. Milk Technol., *4*, No. 6: 314. Nov.–Dec., 1941.

Comparing the microscopic group count, using a binocular microscope with a factor of 600,000 and counting 20 fields with the T.G.M. agar plate count incubated for 48 hours at 32° C., it was found that on Grade "A" raw milk there was a close correlation between the average microscopic and plate counts.

In the case of Grade "B" raw milk, the direct microscopic count was slightly lower than the plate count up to the group ranging between 400,001 to 500,000. In this group the difference was negligible. In the group having counts above 500,000, the miscoscopic count was slightly higher than the plate count.

The results cover a period of two years and were obtained on 4,599 samples.

It was concluded that the microscopic method will give approximately the same results in 20 minutes compared to 48 hours for the agar plate method. L.H.B.

452. Studies of the Resazurin-Rennet Test. (Preliminary Report.)

F. L. SCHACHT AND R. E. NICHOLS, State Dept. Health, Albany, N. Y. Jour. Milk Technol., *4*, No. 5: 281. Sept.–Oct., 1941.

Comparison of the methylene blue, direct microscopic, resazurin and resazurin-rennet tests were made on 730 samples of milk which had passed the odor test.

A total of 3.2% of the samples decolorized methylene blue in two hours or less; 7.1% had a direct microscopic count of 100,000 or over; 18.7% caused a change in color of resazurin in one hour's time; and 36.4% were classed as unsatisfactory with the resazurin-rennet test.

Sixty-one farms were investigated, 44 were found to be unsatisfactory and 17 satisfactory on inspection. The accuracy of the platform tests in detecting unsatisfactory farm conditions were as follows: methylene blue 14%, direct microscopic 23%, resazurin 50%, and resazurin-rennet 89%.

The resazurin-rennet test classified the milk from four farms found satisfactory on inspection as unsatisfactory by test. L.H.B.

453. The Present Status of Homogenized Milk from the Physician's Point of View.

IRVING J. WOLMAN, M.D. The Children's Hospital, Philadelphia, Pa. Jour. Milk Technol., *4*, No. 5: 276. Sept.–Oct., 1941.

Some 800 healthy babies were divided into four comparable groups. One group received unboiled formulas made from milk homogenized by sound waves (sonic vibrations). A second group received formulas made from milk homogenized by low-pressure homogenizers (750 pounds). The third group's formulas were prepared with high-pressure homogenized milk (2500 pounds) and the fourth group (controls) were fed identical formulas made from pasteurized unhomogenized milk which was boiled in the home for five minutes before being used. The homogenized milks were not boiled. All milks originated from the same source were treated in a milk pasteurizing plant under strictly controlled conditions. The making of formulas with homogenized milk was greatly simplified and considerable time was saved.

The study lasted for more than a year and included clinical, laboratory and plant observations.

All babies were placed on the tests on being weaned and were only rarely carried beyond the age of 10 or 11 months, and were kept under careful medical supervision. Generally the children grew normally, the number of gastrointestinal upsets was small, and no appreciable difference was noted with one kind of milk than with another.

"It was concluded that pasteurization and homogenization of whole milk under the conditions of the study resulted in the creation of a milk product possessing soft curd properties and small curd characteristics, features much to be desired in the artificial feeding of infants." L.H.B.

454. We Look at Milk Inspection. J. H. SHRADER, Ed. Jour. Milk Technol., *4*, No. 3: 161. May–June, 1941.

More emphasis should be placed on improved nutrition of dairy products and increasing consumption.

Liberality in the enforcement of milk codes should not mean laxity, but should mean an intelligent tolerance when no subversive principle is involved.

A plea is made for better trained inspectors. L.H.B.

455. How Can Small Milk Producers Meet Pasteurization Requirements? J. H. FRANDSEN, Head, Dept. Dairy Indus., Mass. State Col., Amherst, Mass. Jour. Milk Technol., *4*, No. 3: 158. May–June, 1941.

A brief discussion is given of a number of ways small producers may meet pasteurization requirements. L.H.B.

456. Deaeration as a Means of Retarding Oxidized Flavors and Preserving the Vitamin C of Milk. PAUL F. SHARP, E. S. GUTHRIE, AND D. B. HAND, Cornell Univ., Ithaca, N. Y. Jour. Milk Technol., *4*, No. 3: 138. May–June, 1941.

Also published in Internatl. Assoc. Milk Dealers, Lab. Sect., Proc., Atlantic City, 1940. See JOUR. DAIRY SCI., *24*, No. 9: A252. 1941. L.H.B.

457. An Inventory of Some Methods of Milk Control. WILLARD H. BOYNTON AND IRA V. HISCOCK, Dept. Public Health, Yale Univ., New Haven, Conn. Jour. Milk Technol., *4*, No. 3: 147. May–June, 1941.

As means of increasing the efficiency of laboratory control it is suggested that the standard plate count should be supplemented with the direct microscopic count as a means of determining bacteriological quality—the phosphatase test for determining the degree of pasteurization, and the coliform test for detecting recontamination.

Comparative tests were made to study the relation of direct microscopic counts to agar plate counts, to determine the sensitivity of the phosphatase test, and to determine the thermal resistance of coliform organisms.

The Breed count showed more samples to have high counts than did the plate count, and also indicated that some of the high counts were due to improper handling or to thermophiles.

They found that all the samples of raw milk contained coliform organisms, and that after heating to as much as 140° F. for 30 minutes, no samples gave a positive test for coliforms.

They reported (using Kay and Graham method) that the phosphatase test was consistent in its findings and that the addition of 0.05% raw milk to milk pasteurized at 145° F. for 30 minutes could be detected. L.H.B.

458. What Does It Cost to Produce Milk? H. C. M. CASE, Col. Agr., Univ. Ill. Milk Dealer, *31*, No. 6: 36–46. Mar., 1942.

•Data are presented showing the cost of producing milk in the Chicago and the St. Louis milk-producing areas. The data show that in the northern Illinois area the net cost per 100 pounds of milk averaged $1.72 on 99 selected farms. On the 33 farms with lowest milk cost it averaged $1.44 and on the 33 farms with highest milk cost it averaged $2.11 per hundred. In the St. Louis area the net cost of 100 pounds of milk averaged $1.60 on 110 selected farms. On the 37 farms with lowest milk cost it averaged $1.33 and on the 37 farms with highest milk cost it averaged $1.99 per hundred pounds. C.J.B.

459. Industrial Milk Service. H. W. AMUNDSEN, Chicago Sales Represent., Ideal Dairy Dispenser Co., Bloomington, Ill. Milk Dealer, *31*, No. 6: 31, 60–65. March, 1942.

The tangible benefits of between-meal milk service are listed. These are then classified under the following three types:

1. Better general health, as has been shown by more regular attendance at work. 2. Greater efficiency, as has been shown by more work accomplished in a given period of time. 3. Improvement of morale, as has been shown by employees' attitude toward their work.

The advantages of the vending machine method of milk distribution in defense and other industrial plants are:

1. Milk is kept cold and fresh at all times. 2. Eliminates danger of the dairy-man being injured while working through the plant. 3. Reduces opportunity for the dairy-man to see confidential work on line, bench, or machine and lessens opportunity for sabotage. 4. Eliminates time lost by the dairy-man and employees. 5. Contributes to national defense program by enabling dairy to serve more men, in less time, thereby conserving tires, tubes, gasoline, and oil.

The discussion is partially summarized as follows:

With our industrial activity rapidly gaining momentum, in an *all out for victory at any cost* program, the question of employee health, efficiency, and morale cannot be too strongly emphasized.

To that end, industrial leaders say that milk consumption by workers, between meals, pays tangible dividends in the form of less loss of time due to illness, more cheerful cooperation on the part of the workers, and a general stepping-up in production.

Scientists and medical authorities say that milk is indispensable to the defense worker. Army and navy officials recommend it "by example." Statistics show that 17 *industrial fighters* are required to keep each front line fighter equipped, armed, and fed.

It naturally follows, therefore, that any curtailment in the availability of an ample supply of milk for the defense and other industrial workers, will vitally affect, if not seriously endanger, the very keystone of the defense program, *i.e., production of ships, tanks, guns, planes,* and the necessary munitions to make them effective. C.J.B.

460. Every Other Day Delivery. EDWARD THOM, Assoc. Ed., Milk Dealer. Milk Dealer, *31*, No. 6: 26–27, 74–75. March, 1942.

The author discusses every other day delivery as a practical means of conserving tires on retail delivery trucks as reported by milk dealers throughout the country who have adopted the plan. A saving of from approximately 40 to 60% in mileage is reported. Practically all the dealers are finding the plan well accepted by customers and employees. As a rule no employees are discharged. Some dairies are dividing the routes so as to carry the same load over half the route one day and over the other half the following day. Other dairies are doubling the load and placing two men instead of one on the route. The latter plan is usually discontinued after the men learn the route and the extra men absorbed in the plant or for collections, etc. Several dealers believe that every other day delivery is here to stay. C.J.B.

461. Promotional Work in Milk Control. J. R. JENNINGS, Milk Sanit., Ia. State Dept. Health, Des Moines, Ia. Jour. Milk Technol., *5*, No. 1: 41. Jan.–Feb., 1942.

Some pointers are given on selling milk control to the industry and the community. It is the opinion of the author that a sales reason for improving a particular condition on a farm or in a plant will often prove more valuable in accomplishing the end than will giving the public health reason why it should be done. L.H.B.

PHYSIOLOGY

462. Studies on Absorption from the Rumen. A. D. RANKIN AND H. M. DUKES, Dept. Physiol., N. Y. State Vet. Col., Cornell Univ., Ithaca. Fed. Amer. Soc. for Expt. Biol., Fed. Proc., Part II, *1*, No. 1: 70. 1942.

"In ten experiments with two sheep having permanent rumen fistulas, dextrose was placed in the posterior ventral sac of the rumen. A marked rise in the blood sugar level always resulted. The increases over the level at the time of administration varied from 34% to more than 400%.

"Other experiments revealed that potassium iodide, pilocarpine, strychnine, and sodium cyanide can all pass through the mucosa of the rumen. These experiments lend strong support to the growing belief that the rumen is an organ of absorption." D.E.

463. The Humoral Nature of the Factor Causing the Let-down in Milk. W. E. PETERSEN AND T. M. LUDWICK. Div. Dairy Husb., Univ. Minn. Fed. Amer. Soc. for Expt. Biol., Fed. Proc., Part II, *1*, No. 1: 66. 1942.

"Evidence is presented that the factor causing the let-down of milk is humoral in nature. Surviving bovine mammary glands, perfused with blood from the donor, were cannulated to drain them of all the milk that would drain out. After the milk ceased flowing and with the cannulae *in situ* a liter of blood drawn from cows that had been stimulated to let-down their milk was introduced as the perfusate. Within 15 seconds after the introduction of such blood, the milk flowed copiously out of the cannulae. Blood from cows that were not stimulated to let-down their milk had no effect. Blood from cows that were markedly excited did not cause a let-down of milk but had pronounced vaso-constricting properties reducing the blood flow to as little as one-fourth normal.

"When the blood was permitted to stand for one-half hour, the humoral agent for the let-down of milk was destroyed. In *in vivo* experiments, it was found that the intravenous injection of adrenalin interfered with the let-down of milk even when the injections were made after the cows had been stimulated to let-down their milk. Fright or excitement had similar effects to those of adrenalin." D.E.

MISCELLANEOUS

464. Detergents in the Dairy Industry. CHARLES SCHWARTZ, Hall Labs., Inc., Pittsburgh, Pa. Jour. Milk Technol., *4*, No. 5: 258. Sept.–Oct., 1941.

The various cleansing agents and their uses in the dairy are discussed.

It is concluded that the best detergent to use is one that contains an efficient calcium-sequestering material to prevent or control precipitation or formation of scale and milk stone. It must also contain an alkali sufficient in amount or strength to do the cleansing job at hand, and yet it must be of a type that will be least harmful to both the user and the equipment.

L.H.B.

465. Corrosion Tests on Acid Cleansers Used in Dairy Sanitation. M. E. PARKER, Beatrice Creamery Co., Chicago, Ill. Jour. Milk Technol., *5,* No. 1: 37. Jan.–Feb., 1942.

A report of a laboratory study of the corrosive action of an acid cleansing product known as Mikro-San (reported to be a non-toxic mixture of certain organic acids, specific wetting agents, corrosion inhibitor, and a microstatic agent), an acid sterilizing product known as Mikro-Puer (content not given), and Chicago tap water on nine different metals normally used in the construction of can washers and dairy processing equipment, as well as that used in the construction of milk cans.

The results were compared with those reported by Hunziker, Cordes and Nissen (JOUR. DAIRY SCI., *12:* 140–179 and 252–284, 1929).

It was concluded that the corrosive effects of Mikro-San, Mikro-Puer and Chicago tap water were relatively mild compared to the various mineral and organic acids, and particularly to the action of the various washing powder solutions used by Hunziker, *et al.* L.H.B.

466. Report of the Committee on Sanitary Procedure. Internatl. Assoc. Milk Sanit. for 1941, W. D. TIEDEMAN, Chm. Jour. Milk Technol., *5,* No. 1: 29. Jan.–Feb., 1942.

This report gives a complete list of items which have been accepted during the past three years by the three committees of the Internatl. Assoc. of Milk Sanitarians, the Internatl. Assoc. of Milk Dealers, and the Dairy Industries Supply Assoc., and which have been called the "Three Association Standards."

Specifications for sanitary motors adopted by this committee and one from the National Electrical Manufacturers' Assoc. on April 8, 1941, are also given in detail.

"These specifications are intended to apply to motors as made available for new equipment and are not intended to apply to motors to replace serviceable ones now in use." L.H.B.

467. Report of Committee on Sanitary Procedure. Internatl. Assoc. Milk Sanit., W. D. TIEDEMAN, Chm. Jour. Milk Technol., *4,* No. 4: 214. July–Aug., 1941.

The committee has accepted the following nine items after due investigation:

1. A 14 R ferrule for ground joints. 2. A 15 R ferrule for ground joints. 3. A 15 R ferrule for gasketed joints. 4. A 13 H hex nut. 5. A 16–A ground seat cap. 6. A 3 A interchangeable thermometer fitting for application to vats and pipe lines. 7. "No finish on stainless steel below a number four is acceptable for surfaces in contact with fluid milk products." 8. "Strainers are to have holes of 0.0625 inch diameter in 16 or 18 U.S. gauge metal with selvedge edges of one-half inch or more." 9. The standard 11–C 3-way valve shall have the handle point in the direction of flow with the flow entering at the side opening." L.H.B.

468. After Winning the War—We Must Win the Peace. W. D. McFarlane, MacDonald Col., McGill Univ., Montreal. Canad. Dairy and Ice Cream Jour., *21,* No. 2: 19. 1942.

This article is based on a report of the National Farm Chemurgic Committee of the Canadian Chamber of Commerce. It surveys the factors responsible for many of the economic agricultural problems leading up to the war and points to some of the things which must be put into operation now and following the end of the war. It is the author's opinion that agriculture can surmount its difficulties only through more extended research. The utilization of the forces of agriculture, industry and science to expand rural life should have a tendency to open up new markets and so achieve an equilibrium between farm and industrial incomes which would have a degree of permanency. O.F.G.

469. Good Public Relations Needed by the Dairy Industries. H. W. Comfort, The Borden Co., New York. Canad. Dairy and Ice Cream Jour., *20,* No. 12: 23. 1941.

An appraisal of policies and practices is the first fundamental step in public relations. Correction of the lack of public knowledge of facts is the second fundamental of public relations. Public relations is defined as an "operating program in which management recognizes its social responsibilities, and discharges them, and reports in full to the public so that comprehension and confidence in the organization may be established." The public seems to want from a business organization the same things it expects of an individual member of a community. Good relations with milk producers and company employees is an important feature of good general public relations. O.F.G.

470. Corrosion of Metals—A Review of Some of the Causes and Methods of Prevention. G. G. Marvin, Mass. Inst. Technol., Cambridge.

Internatl. Assoc. Milk Dealers, Assoc. Bul., *34*, No. 8: 165. Jan., 1942.

The importance of corrosion has been recognized only since about 1920. The electro-chemical theory is the one now generally accepted. This is that when metallic iron is placed in water the solution pressure of the iron causes it to dissolve until a state of equilibrium is established between the ferrous ions and metallic iron. Chemical reactions depicting this process are given and a list of primary and secondary factors involved. Six forms of corrosion are described.

Corrosion-preventive measures available include: 1. Controlling the composition of the metal. 2. Protective coatings and passivation. The latter means using inhibitors such as chromates, silicates, phosphates, etc., or a stabilized passive state such as stainless steel. Lists are given of activating and passivating conditions for 18–8 stainless steel. This metal is subject to severe deep pitting which makes it unsatisfactory for some purposes. "Clad" metals in which the surface consists of 10–20% of the protective metal seem a good solution at the present time. This is very economical of the protecting metal, of great importance as in the present metal shortage. Silver plating has become of economic importance and this yields a surface very resistant to corrosion, giving negligible metallic contamination to foods and greater resistance to alkalies or cleaning agents than tin. Glass-coating equipment gives a very corrosion-resistant surface. When using a paint the surface must be well cleaned or possibly chemically dipped.

Prevention of corrosion by means of passivation is especially important with boiler and steam production problems. Here inhibitors bring about a surface condition which retards corrosion. Chromates in brine solution, silicates and phosphates in water are examples. E.F.G.

471. **The Angle Sanitary Fitting.** PAUL F. SHARP, Cornell Univ., Ithaca, N. Y. Jour. Milk Technol., *4*, No. 3: 144. May–June, 1941.

A new type of angle fitting for sanitary piping is described and illustrated. It has many advantages over the old type. L.H.B.

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Burgwald, L. H.
Bushnell, L. D.

Cole, W. C.
Cone, J. F.
Coulter, S. T.

Doan, F. J.

Dorsey, L. M.
Downs, P. A.

Erb, J. H.
Ely, Fordyce
Espe, D. L.

Frazier, W. C.
Fuller, S. A.

Garrett, O. F.
Glick, D. P.
Goss, E. F.

Huffman, C. F.

Irvine, O. R.

Josephson, D. V.

Lucas, P. S.
Lush, J. L.

Marquardt, J. C.
Martin, W. H.
Mueller, W. S.

Price, W. V.

Reece, Ralph P.

Riddell, W. H.

Sheuring, J. J.
Stebnitz, V. C.

Thomsen, L. C.
Trout, G. M.

Webb, B. H.
Weckel, K. G.
Weiser, H. H.
White, G. C.

Yale, M. W.

JOURNALS

American Butter Review
American Milk Review
American Journal of Diseases of Children
American Journal of Physiology
American Journal of Public Health
Archives of Pediatrics
Australian Journal of the Council for Scientific and Industrial Research

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Biochemische Zeitschrift

Canadian Dairy and Ice Cream Journal
Canadian Public Health Journal
Certified Milk
Cornell Veterinarian

Dairy Industries
Dairy World
Deutsche Molkerei Zeitung

Endocrinology

Food Industries
Food Manufacture
Food Research

Ice and Refrigeration
Ice Cream Field
Ice Cream Review
Ice Cream Trade Journal
Industrial and Engineering Chemistry

Journal of Agricultural Research
Journal of Agricultural Science
Journal of American Medical Association
Journal of American Veterinary Medical Association
Journal of Animal Science
Journal of Bacteriology
Journal of Biological Chemistry
Journal of Dairy Research
Journal of Dairy Science
Journal of Endocrinology
Journal of Experimental Medicine
Journal of General Physiology
Journal of Genetics
Journal of Heredity
Journal of Industrial and Engineering Chemistry
Journal of Infectious Diseases
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Oil and Soap

Pacific Dairy Review
Proceedings of Society of Experimental Biology and Medicine

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Scientific Agriculture
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Tierernährung
Tierzüchter

Zeitschrift für Infektionskrankheiten Parasitäre Krankheiten und Hygiene der Haustiere
Zeitschrift für Physikalische Chemie, Abt. A and B
Zeitschrift für Untersuchtung der Lebensmittel
Zeitschrift für Züchtung. Reihe B. Tierzuchtung und Zuchtungbiologie
Zentralblatt für Bacteriologie
Züchtungskunde

SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment. Liebefeld, Berne, Switzerland

International Association of Ice Cream Manufacturers
International Association of Milk Dealers

National Institute for Research in Dairying, Reading, England
New York Association of Dairy and Milk Inspectors

Prussian Dairy Research Institute, Kiel, Germany

State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

BOOK REVIEW

472. Minerals in Nutrition. Z. T. WIRTSCHAFTER, Reinhold Publishing Corp., 330 W. Forty-Second St., New York. 14 Chapters including summary, plus index, 175 pages; $1.75.

Milk is the source of so many important nutritive factors that having a well rounded knowledge of their properties is often left as a duty of the specialist. As a result, the average worker in the dairy industry knows something about the high-lights of the nutritional properties of milk, but more often little about the properties that frequently are important but overlooked. As an example, the abundance and role of the calcium and phosphorous of milk are known even by the consumer; but how many dairymen know about the occurrence and body need and use of the other minerals in milk, such as sodium chloride or potassium, or sulfur? Minerals in Nutrition is a very interesting popularized discussion on the source, function, disposal in the body, effect of excess or inadequate amounts, metabolism, and organic relationships of the many minerals that have specific functions necessarily derived from our foods. Further, the adequacy of milk in providing the quantities of the various elements is cited. The discussions of Minerals in the Body, Action and Distribution of Minerals, What Salt Does in the Body, Potassium, Calcium, Magnesium, Phosphorous, Sulfur, Iron, Iodine, Other Minerals, Pregnancy, and Lactation are short chapters some 6 to 10 pages each filled with simply presented modern information that will be of interest to sales managers as material for their salesmen, to laboratory and nutrition workers, teachers and students.

The information is presented principally in non-technical readers digest arrangement, and is convenient to read, and easy to comprehend.

K.G.W.

BUTTER

473. A Study of the Quality of Retail Butter in Michigan. I. A. GOULD. Mich. Quarterly Bul. *24*, No. 4: 298. 1942.

The butter studied was obtained directly from retail outlets in the cities of Lansing, East Lansing, Flint and Detroit. The samples were scored for flavor and tested for yeast and mold, mold mycelia, extraneous material, and fat content.

A summary of the results shows that: 1. 45.2% of the samples scored under 89; 2. 75.5% had a yeast and mold count over 20 per ml.; 3. 43.3% had a mold mycelia count over 20; 4. 30.2% had a sediment score of 8 or less; 5. 10.2% contained less than 80% fat.

The results indicated no definite relationship between flavor of butter and price, nor between claims on the package and flavor. It is the author's belief that a butter grading system as a means of protecting the consumer is desirable. P.H.T.

CHEESE

474. A Practical Moisture Test for American Cheddar Cheese. I. A. Gould, Mich. Quarterly Bul. *24*, No. 4: 318. 1942.

The oil-salt method described is credited with the following advantages over the procedures commonly used: 1. No special apparatus is necessary; 2. Simple to perform; 3. Can be completed in 20–30 minutes.

The recommended procedure of the oil-salt method is as follows: 1. Reduce sample to particles the size of wheat kernels; 2. To an aluminum moisture pan (2–3 inches deep and 2½–3½ inches wide) add 20 ml. oil (olive or cotton seed) for a 5 gram cheese sample and 25 ml. for a 10-gram sample; 3. Add about 1 gram of common salt; 4. Heat oil-salt mixture over flame until fuming occurs and then cool; 5. Balance pan on butter moisture balance; 6. Weigh 5 or 10 gram sample; 7. Tilt pan to make sure all cheese particles are covered with the oil; 8. Heat pan slowly until all bubbling ceases; 9. Cool; 10. Return pan to scales and determine moisture. If a 5-gram sample was used, double the reading. P.H.T.

475. How to Make Cheese Spreads. C. R. Barker, Chicago, Ill. Natl. Butter and Cheese Jour., *33*, No. 5: 14. May, 1942.

Cheese spreads are combinations of cheese with condensed whey, whey powder, condensed or dry skimmilk or combinations of whey and skimmilk. Spreading qualities are obtained from high moisture or fat. Low-fat "Cream" cheese without rennet is a common base and detailed instructions are given for making it. The acidity of this base is adjusted in the kettle with citric or phosphoric acid or with sodium bicarbonate. Condiments or such cheeses as Limburger, Camembert or Blue cheese are added. Sometimes emulsifying salts and gum stabilizers and buffers are used. Formulas are given for making vinegar-sugar solution; gum paste; Pimento cream spread; Limburger cheese in glass; Special American cheese spread; Relish spread; smoked cheese in sausage casing; and "Nippy"-type cheese. W.V.P.

476. Paying for Quality. Anonymous. Natl. Butter and Cheese Jour., *33*, No. 5: 17. May, 1942.

The Burr Oaks Cheese Factory, Columbus, Wis., grades milk by methylene blue test into two classes, A and B. For B grade milk, 10 cents per hundred is deducted from the regular factory price and prorated among

A grade patrons. If penalties in one month exceed 10 cents per 100 lbs. of A grade then the balance goes to a pool to be paid in December according to average yearly quality tests. Blue tests are made at least once each week and patrons told results immediately. W.V.P.

CHEMISTRY

477. **The Isolation of Biotin from Milk.** Donald B. Melville, Klaus Hofmann, Eleanor Hague, and Vincent du Vigneaud, Dept. Biochem., Cornell Univ. Med. Col., New York City. Jour. Biol. Chem., *142,* No. 2: 615. 1942.

A method for the isolation of pure crystalline biotin from a milk concentrate furnished by S.M.A. Corporation is described. The yield of biotin was 25 to 40% and the method is suitable for the preparation of relatively large amounts of pure biotin. V.C.S.

478. **Does Hydroxyglutamic Acid Occur in Milk Proteins?** Ben Nicolet and Leo A. Shinn, Div. Nutr. and Physiol., Bur. Dairy Indus., U. S. Dept. Agr., Washington. Jour. Biol. Chem., *142,* No. 1: 139. 1942.

The work of these authors tends to show that the values given by earlier investigators for the amount of hydroxyglutamic acid in milk are much too high and it is even doubtful if milk contains any hydroxyglutamic acid.
V.C.S.

DISEASE

479. **The Udder Infusion Treatments in a Herd Program for the Control of Streptococci Mastitis.** C. S. Bryan and H. H. Ruhland. Mich. Quarterly Bul. *24,* No. 4: 290. 1942.

The udder infusion treatments for mastitis which principally involve the use of tyrothricin, novoxil, and acriflavin, have been reported to yield recoveries in 80 to 90% of the cases treated. The dairyman, however, should be acquainted with the following facts before he subjects his cows to treatment: 1. It should be determined by laboratory methods whether or not the infection of the udder is due to streptococcic mastitis; 2. Cows in the advanced stages of the disease should be slaughtered; 3. Cows suffering from an acute attack should be treated after the acute stage of the disease has subsided; 4. Due to the effect of the treatment on milk flow it is best to treat cows near the end of their lactation period or while they are dry; 5. The appearance of the milk may be changed as a result of the treatment and should not be used for human consumption during the period it is of abnormal appearance; 6. The treatment does not confer any immunity to the animal even though a cure results. P.H.T.

480. Feeding as a Contributory Factor in the Development of Chronic Mastitis. EARL N. MOORE, H. O. HENDERSON, A. H. VAN LANDINGHAM, AND CHAS. E. WEAKLEY, JR., West Va. Agr. Expt. Sta. Amer. Jour. Vet. Res. *3*, No. 7: 154. April, 1942.

A group of cows was fed a ration of alfalfa hay, silage and pasture with a grain supplement of corn products at the rate of 1 lb. to 3.5 lbs. of milk. The control group received a ration of mixed grains at the same rate. Each grain ration contained approximately 12.5 per cent digestible crude protein. A second group received the same grain ration as the control group above, half receiving 1 lb. to each 3.5 lbs. of milk and the others only a limited amount.

All cows were handled together, but those free of mastitis were milked first. "No appreciable increase in the incidence or severity of mastitis was observed as a result of the kind of ration or the rate of grain feeding under the conditions of these experiments." S.A.F.

481. Concise Methods for the Detection of Streptococci of Lancefield Group B or C in Milk Samples. EDWARD J. FOLEY, Labs. Bact., Univ. Notre Dame, Notre Dame, Ind. Jour. Milk Technol., *5*, No. 2: 94. Mar.–Apr., 1942.

A laboratory method for the detection of bovine mastitis caused by streptococci of Lancefield Group B or C is described. The method employs the Hotis test and Edward's broth which have been modified by the addition of selective inhibitory agents. The procedure used permits the determination of the presence of *S. agalactiae* and *S. dysagalactiae* without the necessity of preliminary plating and pure culture study.

The modified Hotis test used was as follows: 20 ml. (composite) samples of milk are drawn into sterile tubes containing 1 ml. of a solution made up of sodium azide, 1 gram; brom cresol purple, 5 grams; crystal violet, 0.1 gram; distilled water, 1 liter.

The sodium azide inhibits the growth of *Esch. coli,* while the crystal violet is effective in inhibiting the growth of micrococci. The use of these agents permits prolonged incubation of tubes when necessary.

Modified Edward's broth (composition as follows: lactose, 5 grams; crystal violet 1% solution, 1 ml.; sodium azide, 0.1 gram; tryptose phosphate broth (Difco), 1,000 ml.; final pH 6.8–7.0. Sufficient brom thymol blue is added to impart a slight but distinct color to the broth) is inoculated with a loopful of cream from a modified Hotis test after 24–48 hours incubation. (In routine work the author stated that he only inoculates broth with cream from those tubes which are suspicious.) The broth tubes are incubated at 37° C. "Growth of *S. agalactiae* is manifest within 24 hours as soft or granular floccules, discrete in the clear broth. (This appearance is for all practical purposes diagnostic of *S. agalactiae* and further investigation is usually not required.) *S. dysagalactiae* develops a cloudy growth with more or less sedimentation. With both types the pH falls to 5.0 or below. The volume of work connected with serological identification can be reduced by making films for microscopic examination from broths showing an acid reaction and diffuse turbidity. Only tubes with copious flocculent growth or those shown to contain streptococci by microscopic examination are tested serologically. Those in which growth is not extensive are reincubated and tested the next day if streptococci are demonstrated in the films."

L.H.B.

482. The Role of Disinfectants in the Control of Mastitis.

C. S. BRYAN, C. W. DARBY, W. L. MALLMAN AND A. C. CORBETT, Sect. Bact., Mich. Agr. Expt. Sta. Jour. Milk Technol., *5*, No. 2: 77. Mar.–Apr., 1942.

Eleven strains of streptococci isolated from mastitis-infected udders and laboratory strains of *Staphylococcus aureus* and *Echerichia coli* were used to test the germicidal properties of various soaps.

One per cent concentrations of the various soaps in a 5.0% milk suspension were tested at room temperature to determine their killing action in one minute's time. The following soaps were tested: "Lifebuoy," "Neko," "Ivory Soap" and "Flakes," "Super-Suds," "Fels-Naphtha" Soap Chips, "Oxydol," "Rinso," "Lux Flakes," and "Drene" (a sulphonated alcohol). It was concluded that soap solutions and sulphonated alcohols are not satisfactory agents for destroying organisms causing mastitis which may be on the udder. "Hypochlorite solutions containing 1.0 ppm. available chlorine killed alpha, beta and gamma streptococci in less than one minute."

The test showed that 5.0% milk added to a sodium hypochlorite solution containing 200 ppm. of available chlorine reduced the chlorine content to 51.4 ppm. and that this solution did not kill all strains of streptococci in one minute's time. L.H.B.

483. Mastitis and the Plate Count of Milk. II. The Influence of *Streptococcus Agalactiae* upon the Standard Plate Count of Milk.

MAX E. MORGAN, E. O. ANDERSON, AND W. N. PLASTRIDGE, Depts. of Dairy Indus. and Anim. Dis., Univ. Conn., Storrs, Conn. Jour. Milk Technol., *5*, No. 2: 67. Mar.–Apr., 1942.

Samples of mixed milk from 93 herds known to have harbored animals infected with *Str. agalactiae* were plated in triplicate in Edward's medium and in both new and old A.P.H.A. media.

The results showed that 78 of the samples had beta-hemolytic *Str. agalactiae* present. Fifteen of the samples did not show any of these organisms as being present in three 0.1 ml. portions when plated on Edward's medium.

There was no correlation between the total plate counts and the *Str. agalactiae* counts.

It was found that milk from herds showing a greater percentage of infected quarters gave higher *Str. agalactiae* counts. The new standard medium supported an average of 10.3% more colonies than did the old standard medium.

Grade B producers will seldom be penalized for excessive plate counts due to the presence of *Str. agalactiae* mastitis in the herd. Grade A or certified producers are more likely to be affected. According to the standards for the State of Connecticut, if the *Str. agalactiae* counts of each of the 78 samples showing these organisms on Edward's medium were subtracted from the standard agar plate count, it would have changed the grade in only four cases. Three samples would have changed from Grade B to Grade A and one sample from below legal standard to Grade B. L.H.B.

FEEDS AND FEEDING

484. Grass Silage—Feeding Results. C. B. BENDER, N. J. Agr. Expt. Sta., New Brunswick, N. J. Internal. Assoc. Milk Dealers. Assoc. Bul. *34,* No. 14: 309–313. Feb., 1942.

A grass silage fed as a part of the total roughage may replace all the corn silage and part of the hay. A minimum of 6 lbs. of hay seems desirable for milking cows.

Results of 4 years of growth experiments with Guernsey and Holstein heifers gave 8% less than normal gains in silage winter feeding but greater than normal growth on summer pasture feeding. The average for the 4 years was an average Guernsey gain of .91 lb. against Ragsdale's Standard of .9 and an average Holstein gain of 1.17 against a Ragsdale Standard of 1.2. Normal milk production was maintained when feeding 50–70 pounds of good legume or a mixture of legume and grass silage to Holsteins. Hay consumption was limited to 6–10 lbs. per day. Comparing 3 kinds of silage it was found that the ratio of milk to digestible nutrients value was 1.33 : 1 for molasses silage, 1.36 : 1 for phosphoric acid silage and 1.37 : 1 for corn silage. Milk pasteurized and held for 2 days scored 22.3 when alfalfa silage was fed, 20.8 for corn silage and beet pulp 21.0. The milk produced from feeding grass silage showed much greater resistance to the development of oxidized flavor than milk produced on either corn silage or beet pulp.

E.F.G.

485. Certain Relationships of Avitaminosis A to Vitamin C in the Young Bovine. P. D. BOYER, P. H. PHILLIPS, W. D. POUNDEN, C. W. JENSEN, I. W. RUPEL AND M. E. NESBIT, Dept. Biochem., Univ. Wis., Madison, Wis. Jour. Nutr., *23,* No. 5: 525. May, 1942.

Eleven calves, 8 Holsteins and 3 Guernseys, were used in this study. The blood plasma vitamin A values of the calves not getting vitamin A supplements decreased to a low level of 0.03–0.05 micrograms per cent after two weeks. Papilledema did not develop until after an average of 5 weeks on experiment and increased intracranial pressure did not occur until an average of 8 weeks after low levels of blood vitamin A were observed.

Pathology due to vitamin A deficiency appeared only after the blood plasma levels of vitamin A have decreased to a low level of 0.05 to 0.07 micrograms per cent or less and remained there for several weeks.

The increased intracranial pressure observed in calves suffering from vitamin A deficiency is paralleled by a marked decrease in the ascorbic acid content of the cerebrospinal fluid. Administration of ascorbic acid to vitamin A-deficient calves resulted in a rise in the ascorbic acid content of the cerebrospinal fluid and a reduction in the cerebrospinal pressure in 3 out of 4 calves.

Reduced urinary excretion of vitamin C in vitamin A deficient rats indicated that the lowered blood and tissue vitamin C is the result of impaired synthesis. C.F.H.

FOOD VALUE OF DAIRY PRODUCTS

486. The Increasing Interest in Fortification of Foods with Vitamins and Minerals.

C. A. Elvehjem, Univ. Wis., Madison. Internal. Assoc. Milk Dealers. Assoc. Bul. *34*, No. 11: 249–255. Jan., 1942.

It is suggested that in addition to extending our knowledge with respect to the chemistry of our diet it is necessary that we learn how to apply these newer findings to practical nutrition and that much of the application will continue to be built around milk. A brief review of the history of nutritional discoveries is given with emphasis upon the various vitamins. The rough and tedious methods of biological assay are being supplemented or replaced by chemical methods and more recently by bacteriological methods in the case of nicotinic acid. In spite of cheap vitamin preparations the author believes that the safest program is to rely upon the common foods as a source of vitamins. Since milk is usually used with fruit juices and refined cereal products in the infant dietary the two deficiencies most likely to appear are vitamin B_1 and nicotinic acid. On this account dairymen should be particularly interested in the enriched bread program. Being a good source of riboflavin milk is useful in conjunction with enriched bread which is low in this vitamin. Milk is a good source of B_6 and pantothenic acid. Milled wheat has lost five-sixths of its thiamin and nicotinic acid but only about half of its B_6 and pantothenic acid. It is more important to fortify the average diet with milk than it is to fortify milk. The results of a nutritional program come slowly and we shall see the greatest effects in the next generation. E.F.G.

487. Relation of Volume of Dairy Milk Production to Ascorbic Acid Content of Cow's Milk. A. D. HOLMES AND FRANCIS TRIPP, E. L. Patch Co., Boston, Mass., E. A. WOELFFNER, H. P. Hood and Sons, Boston, Mass., AND G. H. SATTERFIELD, Univ. N. C., Raleigh, N. C. Food Research, 7, No. 2: 111. Mar.–Apr., 1942.

An investigation of the ascorbic acid (reduced) content of cow's milk was conducted over one year using Guernseys and Holsteins. It was found that the volume of milk produced per day has little influence on the ascorbic acid content but that as the volume decreases the total output of ascorbic acid in the milk drops.

The data indicate that Guernsey milk is slightly higher in ascorbic acid content than Holstein milk. Also that the lowest ascorbic acid level for Guernsey milk was found in milk from animals producing less than five liters of milk per day whereas the highest ascorbic acid level for Holstein milk was from animals producing less than five liters of milk per day. Too few animals were included in the latter groupings, however, to make this comparison conclusive. F.J.D.

488. Riboflavin Content of Some Common Foods. HAZEL E. MUNSELL, Dept. Chem., Columbia Univ., N. Y. Food Research, 7, No. 2: 85. Mar.–Apr., 1942.

Samples of representative foods from different food groups were assayed, by the rat-growth method, for riboflavin. Data obtained were compared with results obtained by other investigators and summarized in a comprehensive table.

Cheddar cheese exhibited the highest riboflavin content, between 545 to 600 micrograms/100 grams. Dried navy beans were also excellent sources. Milk and lean beef, pork and salmon assayed over 200 μ gm. The results with eggs were variable but averaged well over 200 μ gm. Among the leafy vegetables spinach and broccoli leaves gave high values paralleling their greenness. Green string beans, green peas and endive were on a par with milk. The less-green leaves of cabbage and lettuce gave low value as did also peppers even though green. Roots and tubers gave uniformly low values and cereals were intermediate.

Some factors affecting the precision of the rat-growth assay method are discussed. F.J.D.

ICE CREAM

489. A Comparison of Four Ice Cream Stabilizers. W. H. MARTIN AND W. J. CAULFIELD, Kansas State College, Manhattan, Kansas. I.A.I.C.M. Prod. and Lab. Council Proc., p. 35. 1941.

Optimum amounts to use of the following stabilizers for ice cream were found to be 0.4% Vesterine (a mixture of mono- and di-glycerides and high test gelatin), 0.35% Kragel, 0.35% of 250 Bloom gelatin, and 0.27% Dariloid. Ether extract, total solids, moisture, color, odor, and pH determinations of the four were made. Gelatin and Vesterine were acid, the other two basic in reaction, as well as characterized by high initial viscosity. Vesterine reduced time required to reach 100% overrun by 18 to 20%. All ice creams made with the various stabilizers withstood two weeks storage equally well. The partial neutralization caused by the basic stabilizers resulted in slightly better body and texture scores, amounting to 0.1 to 0.2 points, although this, while resulting in greater protein stability, had no marked effect on viscosity and exhibited no other advantages. Gelatin and Vesterine stabilized ice creams resisted heat-shock treatment better than the other two. P.S.L.

490. The Influence of Homogenizing Pressures on the Dryness of Ice Cream when Drawn from the Freezer. J. HOFFMAN ERB, Ohio State Univ., Columbus, Ohio. I.A.I.C.M. Prod. and Lab. Council Proc., p. 7. 1941.

Although several factors may operate to produce the desirable dryness sought for in ice cream as it is drawn from the freezer the author confined his study to the effects of homogenization on dryness. Mixes of varying composition were homogenized at varying pressures using single and two stage valves. Mixes homogenized at 1000 pounds were dry in appearance when drawn from the freezer but were partially churned; at 3000 or more pounds pressure they became more wet in appearance; and when rehomogenized the appearance of wetness was greatly increased. This appearance was not in all cases an index of the amount of water frozen in the mix but may be of the thoroughness of fat dispersion. An increase in fat content caused an increase in wet appearance under identical pressures. At 1000 pounds pressures low fat ice creams melted with a curdy appearance and high fat ice creams wheyed off. Smoothness of melting was improved greatly at 3000 or more pounds pressure. Increase in pressure hastened the rate of melting. P.S.L.

491. A Progress Report on a Study of Factors Affecting Shrinkage in Ice Cream. P. H. TRACY, W. A. HOSKISSON AND C. F. WEINRICH. I.A.I.C.M. Prod. and Lab. Council Proc., p. 16. 1941.

Several factors are listed which encourage or discourage shrinkage in a normal 12% fat mix, homogenized in double and single stage homogenizers at usual pressures. Except in one series, paper Sealright containers were used. After hardening, the samples were subjected for 30 minutes to an hour to 25 inches of vacuum and then returned to the low temperature room. An increase in fat, serum solids or sugar increased shrinkage. Shrinkage increased usually in the following order from least to greatest with these sugars: "Frodex," "Sweetose," sucrose, and dextrose; and for these stabilizers: gelatin, sodium alginate, "Hygell," and pectin. Dry egg yolk, monoglyceride, sodium caseinate, high overrun, high sugar content, protein destabilization, smaller ice crystals, smaller air cells, continuous freezing, chocolate liquor, and use of unparaffined paper containers favor shrinkage. Factors found to discourage shrinkage were high drawing temperatures, superheated condensed milk, precipitated protein in condensed milk, exposure to melting temperatures, and manufacture of strawberry and chocolate ice creams, the latter made from cocoa. In the discussion considerable attention is given in the article to ice structure and strength of air cells. P.S.L.

492. Lecithin, Lecitho-Protein, and Glyceryl Monostearate in Ice Cream. K. V. BRYAN, Purdue Univ., Lafayette, Ind. I.A.I.C.M. Prod. and Lab. Council Proc., p. 29. 1941.

The action of lecithin and glyceryl monostearate has been ascribed theoretically to their properties as wetting agents. A normal 12% fat mix was divided into three portions before pasteurization, to one of which was added portions of commercial lecithin varying from 0.15 to 0.50%; to a second, lecitho-protein varying from 0.03 to 0.15%; and to a third, glyceryl monostearate varying from 0.18 to 0.30%. The first two improved body and texture of the ice cream but retarded overrun-incorporation somewhat. The third improved whipping ability but slightly weakened the body. A mixture of the second and third produced a satisfactory ice cream. P.S.L.

493. Sugar Is More Than a Sweetening Agent. ALAN LEIGHTON, Div. Dairy Res. Labs, U. S. Bur. of Dairy Indus. Ice Cream Trade Jour. *38,* No. 5: 14. May, 1942.

In addition to their sweetening value, sugars also expand to a marked degree the temperature range within which the product remains in a palatable semi-frozen condition. Also sugar is sufficiently soluble to remain in solution in the unfrozen water of frozen desserts, except occasionally in water ices. It adds bulk to the unfrozen phase of which volume and viscosity are important for the quality of the product. This highly viscous unfrozen phase holds the air cells, and dilutes and lubricates the ice crystals and the milk solids particles, giving the characteristic smoothness essential to frozen desserts.

To prevent sugar crystallization in sherbets held at low storage temperatures, a certain per cent of dextrose is substituted for part of the sucrose.

Data indicates that dextrose is about 62.5 as sweet as sucrose depending on the concentration of sucrose, while in an ice cream mix it has a sweetening value of 89 compared to sucrose as 100.

Other sugars and syrups may be used to replace part of the sucrose in frozen desserts and invert sugar has also been recommended. In substituting for sucrose it may be necessary to increase the total solid content of the mix and lower freezing and storage temperatures if the ice cream is to remain in a satisfactory condition. W.H.M.

494. Working Directions for Ice Cream Sweeteners. P. S. LUCAS, Mich. State Col. Ice Cream Field *40,* No. 3: 22. 1942.

The author gives a number of ice cream formulas in which sucrose substitutes were used during the last world war, and lists several compilations giving relative sweetness of various sugars and sweetening agents. Considerable variation exists in the relative sweetness of the same sugars as reported by different authors.

Honey is suggested to replace 20% sucrose and using this mix for all flavors without complaint. Amber and light colored honey are recommended as being better than dark colored and strong flavored honey such as buckwheat, sage, and basswood.

In ordinary usage corn sugar has been employed to replace 25% of sucrose but it can be safely used to replace 33%.

Corn syrup, composed of glucose, dextrines and maltose, affects both taste and body. When used in excess of 25%* in the replacement of sucrose it imparts an unpleasant taste. This also increases the smoothness and "chewiness" of the ice cream. Glucose,* a heavy syrup, is little more than ½ as sweet as sucrose and should not be used in excess of 25% of the sucrose according to the author. Invert sugar can also be used to the extent of 25%. Dried corn syrup solids likewise can be used to replace 25% sucrose. Because of cost or low sweetening value few other sweeteners are available to the ice cream manufacturer.

* Abstract editor comment. Many plants are using 25 to 50% replacement with corn syrup without complaints from the trade. W.C.C.

495. Sugar Problems. EVAN R. BOWINS, New State Ice Cream Co., Oklahoma City, Okla. Ice Cream Field *40,* No. 3: 34. 1942.

Sugar is not only a sweetener but it affects (1) body and texture of the ice cream, (2) melting resistance of the finished ice cream, (3) freezing point of mix, (4) time required to freeze, and (5) time required to obtain desired overrun. Besides it has often been used as a cheap source of solids even though the sweetening value was not needed. Since 1924 the sugar content in ice cream has been gradually increased until now we are using 35 to 45 pounds more per 100 gallons of mix.

Mention is made of the use of corn and invert sugars in conjunction with sucrose in ice cream. Figures are given to show that using the method of hydrolysis proposed by Ruehe and assuming invert sugar to be 40% sweeter than sucrose, that a combination of sucrose and invert sugar can be used to give 12% sugar in the mix with a sweetening equivalent of 13.6% compared to sucrose alone. Sugar restrictions in other fields will influence consumer tastes for sweetness in ice cream. W.C.C.

MILK

496. The New Orleans Market for Fluid Milk. R. M. Grigsby and R. A. Ballanger. Louisiana Bul. No. 339. Feb., 1942.

Within the city limits of New Orleans there are 113 small dairies where milk is produced and sold directly to the consumers. Of these, 107 average less than 2 acres each in total land area and have an average of 30 milk cows. Abandoned graveyards, vacant lots, canal and river banks are the principal sources of pasteurage. The most widely used dairy feed is brewery mash. The milk is produced under conditions that resemble a manufacturing process more than normal dairy farming. That part of the supply area further from the city resembles more the normal type of dairy farming. Approximately 265 producer-distributors deliver their milk daily to consumers in the city. This represents 36% of the fluid milk consumed in the market. Most of the producer-distributors handle only unpasteurized milk. Of the total handled by this group, 15% is pasteurized.

Much (86.5%) of the milk in New Orleans is sold wholesale indicating that consumers purchase most of their milk through stores. This is thought to be due to (1) the relatively low per capita income, (2) the high temperatures which prevail throughout most of the year, (3) the lack of refrigeration in many of the homes, and (4) a 2 to 3 cent spread in the price of home delivered and store milk.

Each individual handler (aside from the producer-distributors) pays for his milk according to its use. Consequently there is a variation in the price the farmers receive. There has been an increase in the total amount of milk entering the New Orleans market in recent years. A high class 1 price which encouraged production resulted in little change in the average price received by the farmer due to the increased amounts of milk in class 2 and 3. A high class 1 price has caused some dealers to raise the retail price of milk which resulted in a decreased demand and consequently a greater amount of milk in class 2 and 3. It is questionable whether New Orleans' dairymen can profitably produce milk to be manufactured into butter, cheese and condensed milk. P.H.T.

497. Development of Oxidized Flavor and Speed of Oxidation of Ascorbic Acid in Milk. G. H. Hartman and O. F. Garrett, N. J. Agr. Expt. Sta., New Brunwick, N. J. Jour. Milk Technol., *5*, No. 2: 86. Mar.–Apr., 1942.

In samples of mixed herd milk pasteurized in the College Creamery, it was found that milk was being contaminated with copper from a bronze pump, and the milk developed an intense oxidized flavor by the end of 72 hours. Replacing the pump with a stainless steel one reduced the development of the flavor decidedly but did not prevent it entirely. It was noted that the rate of oxidation of ascorbic acid was coincident with the development of the oxidized flavor. L.H.B.

498. Freezing Cream for Storage. G. M. Trout, Mich. Agr. Expt. Sta., East Lansing, Mich. Milk Dealer, *31*, No. 7: 38–40. April, 1942.

A discussion of the uses for frozen cream and the problems involved in the freezing of cream for storage purposes are presented. A report is then given of the studies made at the Mich. Agr. Expt. Sta. The results of these experiments are as follows: 1. Cream to be stored frozen must be pasteurized at least at an exposure of not less than 165° F. for 15 minutes and preferably 185° for 5 minutes. The high temperature exposure seemed to have a marked effect upon stabilization of flavor, thus inhibiting the development of oxidized flavor. 2. Homogenization had very slight inhibitory action towards oxidation. 3. High carotene content of cream did not stabilize the cream against metal-induced oxidized flavor. 4. Frozen cream of high initial titratable acidity was prone to develop off-flavors upon storage, particularly when pasteurized at 150° F. for 30 minutes. 5. High quality, low-acid cream, free from copper contamination, may be safely stored over an extended period. 6. The type of container in which cream may be stored was of little consequence so far as off-flavor development was concerned. 7. The "oiling off" of frozen cream is affected by rate of freezing, rate of defrosting, fat content, addition of sugar, and homogenization. 8. Only high-fat, excellent quality, low-acid cream, free from copper contamination, should be considered for freezing. C.J.B.

499. Standardization of the Titratable Acidity Test. J. G. Davis and G. M. Sodek, Natl. Inst. for Res. in Dairying, Shinfield, Reading. Milk Indus., *24*, No. 10: 33–35. 1942.

The titratable acidity test is the favorite test in the dairy industry for the detection of souring or incipient souring. In England much of the milk required for liquid consumption has to travel long distances and does not reach the consumer for thirty-six hours or longer. As a result more sensitive and accurate tests are required for assessing the quality of the individual producer's milk.

The titratable acidity test is not an accurate measure of the degree of souring of milk because fresh milk contains relatively no lactic acid and when milk is titrated the very weak acidity of the protein changes to a mild alkalinity. Phenolphthalein indicator does not give a sharp end point in the titratable acidity test.

Since the titratable acidity test is only an arbitrary measurement and has no constant relationship to the lactic acid present, realization must be made, if the test is to be used for a standard of quality in milk, that all details of the test must be standardized. The amount of indicator, method of reading the burette, determining the end point, measuring the volume of milk, carbonation of the soda, and temperature are factors which affect the laboratory results.

The authors recommend the following procedure: Titrate twenty ml. or 20.64 grams of milk with $\frac{N}{10}$ alkali, using 2 ml. of 0.5% phenolphthalein as an indicator. Titrate to a grayish pink color which compares with a control sample held in a similar dish or flask. The determination should be made at a temperature between 55 and 75° F. J.J.S.

500. Recent Canadian Research on the Resazurin Test. C. K. JOHNS, Dominion Dept. of Agr., Ottawa, Canada. The Internatl. Assoc. of Milk Dealers. Assoc. Bul. *34,* No. 11: 256–265. Jan., 1942.

A "triple reading" test was found superior to the "one hour" test in detection of high bacteria and leucocyte counts. This "triple reading" test consisted of using a single color standard (their No. 8 P7/4 Munsell notation) approximately half way between the original blue and the pink. All samples showing a greater color change than this in one hour were placed in 4th grade, those showing a similar change between the 1st and 2nd hours in 3rd grade; those doing the same by the 3rd hour would be in 2nd grade; while the remainder would be 1st grade. This method of reading reflected high leucocyte counts much more definitely than did either resazurin "pink" or methylene blue reduction tests. The dye seems to be sensitive to certain compounds in abnormal milks which do not influence the electrode potential. Sediment was removed from high leucocyte milk by centrifugal force and then added to low leucocyte milk. This was repeated four times. This sediment still hastened the color change which showed that the leucocytes themselves might be a causative agent. The color change is independent of potential and oxygen content. It is suggested that the resazurin test be widely tried out in the industry even though the dye itself may not yet be entirely standardized. E.F.G.

501. An Economic Analysis of Fluid Milk Markets in Indiana. C. M. HARDIN, Purdue Univ., Lafayette, Ind. Bul. *463.* 1941.

In 1940 control board price fixing orders were operating in 22 Indiana fluid milk markets. Three of these were operating under both federal and state orders.

In the period 1937 to 1940 lowest blend prices for milk were paid in the larger markets. At the same time milk receipts in these markets showed no evidence of decreasing.

Larger markets tended to have greater seasonal fluctuation in receipts due to the larger proportion of smaller producers whose seasonal production fluctuates more than that of the larger producers.

The dealers in the small markets attempt to keep the surplus down to a minimum while in the larger markets where a greater volume is handled making efficient manufacturing possible, there is less incentive for keeping surplus milk at a minimum.

In the Indianapolis market if the total receipts had been reduced until 80% instead of 51% of the total receipts were used for class 1 purposes, the retail price of milk could have been reduced ⅞ cent during the 4-year period 1937–1940, without affecting the blend price to producers.

The retail price of milk and dealer's margins were higher in the larger cities.

The blend prices for 4% milk equivalent in the 20 fluid milk markets averaged 35 cents per hundredweight higher than prices paid by condenseries during the 4-year period 1937–1940.

While the amount of milk received during the 1937–1940 period increased, the number of producers decreased.

In 1940 in the 8 markets which operated on the base and surplus plan during the 4 years, daily receipts during the month of highest production were 120% of daily receipts during the month of lowest production. In those markets not operating on the base and surplus plan the receipts during the high month were 149% of receipts during the low month.

A correlation existed between the monthly index of business activity in Indiana and the consumption of fluid milk during the period 1937 to 1940.

P.H.T.

502. The Supply and Utilization of Milk in Indiana. C. M. HARDIN, Purdue Univ., Lafayette, Ind. Bul. *462*. 1941.

Of the 3 billion pounds of whole milk equivalent (4% fat) delivered to Indiana plants in 1939, 57% was in the form of whole milk, 42% in the form of cream, and 1% in the form of farm-made butter. The quantity of cream delivered between 1919 and 1939 changed very little while milk increased from 26% in 1919 to 57% in 1939 of the total receipts.

The utilization of the milk and cream received in 1939 was approximately as follows: Butter 50%; Fluid 22%; Cheese 8%; Condensed 8%; Ice Cream 3.6%; Sweet cream for eastern markets 4.0%; Fluid milk for Chicago 4.0%.

Cheese plants in Indiana average about four times as large as those in Wisconsin. This was thought to be due to the fact that the Indiana plants draw milk from a larger area and most of them have been built since 1926 by large corporations. One-fourth of the cheese plants made half of the total cheese produced in 1939.

The 13 organizations of regional or national scope that operated in Indiana during 1939 handled about half of the combined milk and cream receipts for the state. Five of these organizations handled a third of the total receipts.

The seasonal fluctuation in the receipts of cream from farms is greater than that of milk.

The per capita consumption of fluid milk and cream in Indiana for the year 1939 averaged .60 pint per day. P.H.T.

PHYSIOLOGY

503. **Lactogenic Content of Pituitaries of Pseudopregnant Rabbits.** Joseph Meites and C. W. Turner, Univ. Mo. Proc. Soc. Expt. Biol. and Med., *49*, No. 2: 193. Feb., 1942.

Pseudopregnancy was induced in 11 New Zealand White rabbits by the intravenous injection of 100 I.U. of chorionic gonadotropin. Seven normal, mature, female rabbits served as controls. Twenty days later all animals were killed and their pituitaries were removed for assay. The pituitaries from the control rabbits contained an average of 10.05 Reece-Turner lactogen units per gland and the pituitaries from the pseudopregnant rabbits contained an average of 10.25 R.-T. units per pituitary. It was concluded that the reason no substantial lactation occurs in the rabbit during pseudopregnancy is because the amount of lactogenic hormone in the pituitary is too low. R.P.R.

504. **Extraction and Assay of Lactogenic Hormone in Postpartum Urine.** Joseph Meites and C. W. Turner, Univ. Mo. Jour. Clin. Endocrinol., *1*, No. 11: 918. Nov., 1941.

Two methods were described for preparing postpartum urine for the assay of lactogenic hormone. The first consisted of an alcoholic precipitation and in the second the fresh urine was dialyzed and then concentrated by evaporation. The micro pigeon test was used for the assay of urinary lactogen. The urine of 10 lactating women during the first 2 postpartum weeks revealed a daily range in hormone excretion of from 4.05 to 12.50 international units of lactogen. In 3 cases of definite or suspected hypogalactia less lactogenic hormone was found in the urine than in mothers with adequate lactation. The 3 best lactating women excreted the highest average amount of lactogen in the urine. Pregnancy urine contained only $\frac{1}{8}$ to $\frac{1}{16}$ the amount of lactogen found in postpartum urine. R.P.R.

505. **A Comparison of the Acetone Body Metabolism of the Lactating Mammary Gland of the Normal Cow with that of the Cow with Ketosis.** J. C. Shaw, Dept. Dairy Indus., Storrs Agr. Expt. Sta., Storrs, Conn. Jour. Biol. Chem., *142*, No. 1: 53. 1942.

The lactating mammary gland of the cow in ketosis used over 100% more B-hydroxybutyric acid per 100 cc. of blood traversing the gland than the gland of the normal cow. Simultaneously there appears to be a decrease in oxygen utilization.

Practically all of the oxygen taken up by the lactating gland of the cow with ketosis would be needed for the complete combustion of the B-hydroxybutyric acid removed from the blood by the gland.

In the normal gland only 37% of the oxygen consumed would be required for the complete combustion of the B-hydroxybutyric acid used. It is suggested that much of the energy for milk production in the normal gland is derived from the oxidation of fat in which approximately 37% of the oxygen consumed is used in the oxidation of B-hydroxybutyric acid, while the remaining 63% is used in the oxidation of other fat.

Acetoacetic acid is not utilized by the active mammary gland of either the normal cow or the cow with ketosis. V.C.S.

506. Growth and Development with Special Reference to Domestic Animals. LIII. Resting Energy Metabolism and Ventilation Rate in Relation to Body Weight in Growing Jersey Cattle, with a Comparison to Basal Energy Metabolism in Growing Man. Samuel Brody, H. H. Kibler, and A. C. Ragsdale, Univ. Mo., Columbia, Mo. Mo. Agr. Expt. Sta., Res. Bul. 335. Dec., 1941.

"This bulletin presents charts, prediction tables in various units, and the fitted equation $Y = aX^b$ relating resting-maintenance energy cost, Y, to body weight, X, for the same 18 Jersey cattle from birth to 25 months of age. These results are compared to a similar analysis of energy metabolism (mostly "basal metabolism") in humans from birth on. The value of the exponent *b* in the above equation is near unity from birth to 5 months in cattle and birth to 3 years in children; and near 0.6, from 6 to 25 months in cattle and 3 to 16 years in children. The metabolism per unit surface area rises from birth to about 6 months in cattle and from birth to about 3 years in humans; it remains roughly constant 6 to 25 months in cattle and declines somewhat 3 to 16 years in humans. Similar data are presented for the relation of ventilation rate to body weight in cattle. The value of *b* in the above equation for ventilation rate in cattle is about 0.72, from birth to 25 months. Oxygen decrement in the ventilated air declines with increasing body weight; that is, more oxygen is taken out from a given volume of inspired air in the smaller animals. The implications, practical and theoretical, to students of nutrition and of ventilation and air-conditioning engineering, are discussed." Authors' Abstract

507. Growth and Development with Special Reference to Domestic Animals. LII. Relation Between Organ Weight and Body Weight in Growing and Mature Animals. S. Brody and H. H. Kibler, Univ. Mo., Columbia, Mo. Mo. Agr. Expt. Sta., Res. Bul. 328. May, 1941.

"Detailed charts are presented relating the weights of each of the major

visceral organs to the corresponding body weights in 1) mature mammals of different species; 2) mature birds of different species; 3) animals of the same species, growing and mature. The relative-growth equation $Y = aX^b$ was fitted to each set of data, and the numerical values of the exponent, *b* discussed with reference to the metabolic levels. For mature animals of different species, the weights of the neuro-endocrine system, such as the brain and pituitary gland, which are the metabolism-controlling organs, tend to increase with approximately the same fractional power as does *basal* metabolism; the cardio-respiratory systems, such as the heart, which carries the working burden of the body, tends to increase more directly with body weight than with the *basal* metabolism. The organ-body relations in growing animals vary with the stage of growth; the value of *b* tends to be lower for later growth in the same species than for mature animals of different species. The results are discussed from theoretical and practical view points."
Authors' Abstract

MISCELLANEOUS

508. Man Power and Transport Economics. S. CLIFFORD. Milk Indus., *24,* No. 10: 25. 1942.

Proposals are suggested to prohibit milk customers in England from changing their retailer and to prohibit the misuse of milk bottles by Order during the war. A proposal was made for the standardization of milk churns but that the use of churns with long shoulders be continued as they went a long way to avoid spillage.
J.J.S.

509. Public Health Compliance by Manufacturers of Paper for Packaging Perishable Foods. J. R. SANBORN, N. Y. State Agr. Expt. Sta., Geneva, N. Y. Jour. Milk Technol., *5,* No. 2: 88. Mar.–Apr., 1942.

It has been demonstrated that the growth of microorganisms in paper mills can be effectively controlled. Several large producers of paper board were able to demonstrate that it is possible to produce a board that contains less than 250 colonies per gram.
L.H.B.

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ABSTRACTS OF LITERATURE

BOOK REVIEWS

510. Industrial Waste Treatment Practice. E. F. ELDRIDGE, Research Associate, Eng. Expt. Sta., Mich. State College. McGraw-Hill Book Co., Inc., New York. 1942.

This book is the culmination of years of work on the part of the author in the field of industrial waste treatment and disposal. Many of the problems which arise in connection with the various industrial wastes are of very diversified nature and become complicated when the wastes must be handled through domestic sewage systems. More pressure is being put upon industrial plants to diminish or to entirely eliminate the pollution of bodies of water which are used either as sources of municipal water supplies or for public recreational purposes. The dairy industry is pointed out to be one of the outstanding sources of wastes from its milk processing and manufacturing plants. For example, a milk condensery or a cheese factory may have a waste disposal load equivalent to that of the sewage from a population of 1,500 to 3,000, the number of people in a small town. On the other hand, the combined effluents of a large city's milk plants and ice cream plants can be the source of a heavy waste load on the municipal sewage disposal system.

The chapter listing is as follows: I. Stream Pollution, II. Characteristics of Industrial Wastes, III. Standard Treatment Methods, Structures, and Equipment, IV. Wastes from the Beet-Sugar-Industry, V. Milk Products-Factory Wastes, VI. Canning-Factory Wastes, VII. Tannery Wastes, VIII. Pulp-and-Paper-Mill Wastes, IX. Textile Wastes, X. Meat Packing and Slaughter-House Wastes, XI. Laundry Wastes, XII. Wastes from the Metal Industries, XIII. Gas- and Coke-Plant Wastes, and Other Phenolic Wastes, XIV. Wastes from Fermentation Industries, XV. Wastes from Oil Fields and Refineries, XVI. Treatment of Combined Industrial Waste and Domestic Sewage, XVII. Methods of Analysis for Industrial Wastes.

In Chapter V, Milk-Products-Factory Wastes, the subjects taken up are, Manufacturing Processes and Source of Wastes, Composition of Milk Waste, Preventing Milk Losses, Segregation of Clean Water, Volume and Strength of Waste, Treatment Processes, Standard Biological Filter, Continuous Recirculating Filter, Fill and Draw Recirculating Filter, Biochemical Process, Mallory Process.

This book is recommended as a reference for dairy manufactures and dairy technology students, and to dairy plant executives and milk sanitarians as an authoritative source of information on dairy waste treatment and disposal. L.M.D.

511. The Physiology of Domestic Animals, 5th Edition. H. H. Dukes. Comstock Publishing Company, Inc., Ithca, N. Y. 721 pages, illustrated, $6.00.

Readers of the Journal of Dairy Science who are interested in the production phases of the industry will be interested in learning of the recent revision of this well known and widely used textbook of animal physiology.

We are living in a dynamic world. To be of greatest usefulness textbook recordings of scientific progress must be constantly revised. In recognition of this need "The Physiology of Domestic Animals," first published in 1933, is now in its 5th Edition. During this period, not only has the book been kept up to date but the scope of subject matter covered has been expanded.

For the present edition the entire book has been revised and a good part of it completely rewritten. A number of subject matter additions have been made and the amount of applied material increased. These changes enhance its usefulness as a college text and reference work. T.S.S.

BACTERIOLOGY

512. Inhibition by Phospholipids of the Action of Synthetic Detergents on Bacteria. Z. Baker, R. W. Harrison and Benjamin F. Miller, Univ. Chicago, Chicago, Ill. Jour. Expt. Med., *74*, No. 6: 621–637. Dec. 1, 1941.

This investigation suggests that detergents may be responsible for denaturizing the bacterial proteins or changing the cell membrane in such a way that it affects the metabolism of the bacterial cell.

The addition of phospholipids (lecithin, cephalin, and sphingomyelin) to synthetic anionic and cationic detergents prevents the inhibition of bacterial metabolism.

"The phospholipids must be added either before or simultaneously with the detergent. Addition after the detergent is without effect. Bacteria still exhibit this phenomenon after they have been exposed to the phospholipid and thoroughly washed."

Bactericidal compounds isolated from soil bacteria in the presence of phospholipids show an inhibition of bacterial metabolism. H.H.W.

513. The Bactericidal Action of Synthetic Detergents. Zelma Baker, R. W. Harrison and Benjamin F. Miller, Univ. Chicago, Chicago, Ill. Jour. Expt. Med., *74*, No. 6: 611–620. Dec. 1, 1941.

A study of the type, structure, action on bacterial metabolism, and germicidal properties of 20 cationic and anionic detergents were studied on 6 Gram-positive and 6 Gram-negative micro-organisms.

Zephiran, Phemerol, Retarder LA, Emulsol 605, Catol, Emulsol, 607, Emulsol 660B, Damol and Emulsol 609 are trade names of cationic detergents. Cetyl sulfate, sodium myristyl-sulfate, Duponol LS, Tergitol-8, Triton W-30, Igepon T, Tergitol-7 and Tergitol-4 belong to the class of anionic detergents.

The selected detergents were used in concentrations of 1: 1000, 1: 3000, 1: 6000 and 1: 30,000 at pH 7.0 and with time intervals ranging from 10 to 90 minutes exposure of the various test organisms.

The cationic detergents showed a greater germicidal action on the Gram-positive organisms than on the Gram-negative bacteria.

A relationship appears to exist between the bactericidal action and the chemical structure of the detergent. H.H.W.

BREEDING

514. The Incidence of Right and Left Horn Pregnancies in Dairy and Beef Cattle.

M. Erdheim, U.S.B.A.I., Chicago, Ill. Jour. Amer. Vet. Med. Assn., *100,* No. 781: 343. Apr., 1942.

Observations were made on a total of 3,824 pregnant uteri on the post-mortem table. 1,506 were from dairy cattle and 2,318 from beef breeds.

"In the dairy group, the ratio of right to left pregnancies was 2: 1 and in the beef breeds approximately equal.

"There was one pair of twins to every 89 pregnancies in the dairy groups and one in every 126 for the beef cattle.

"Of the 17 twin pregnancies in both of the dairy groups studied, 15 were bilateral and 2 unilateral. In the beef breeds 16 of the twins were bilateral and 3 unilateral." S.A.F.

BUTTER

515. Diacetyl and Acetylmethylcarbinol Production in the Manufacture of Unsalted Butter.

T. I. Hedrick and B. W. Hammer. Iowa Agr. Expt. Sta. Res. Bul. *301.* May, 1942.

During the ripening of sweet or neutralized sour cream in laboratory and semi-commercial trials there were some irregularities in the effects of various factors on the diacetyl and acetylmethylcarbinol contents of the cream, but generally the contents were increased by an increase in the acidity to which the cream was ripened, by addition of small amounts of citric acid to the cream and by agitation (shaking in the laboratory trials and revolving the coils in the semi-commercial trials) during the ripening. In some trials the contents were greatly influenced by the use of certain butter cultures, while in other trials they were not.

In general, as the diacetyl contents increased in the ripening cream the acetylmethylcarbinol contents also increased, but there were variations from this relationship. The occasional decreases in diacetyl contents often were accompanied by increases in acetylmethycarbinol contents.

Some of the ripening procedures used with the cream were beneficial from the standpoint of score of the butter under certain holding conditions. These procedures included development of higher acidities in the cream, addition of citric acid to the cream and agitation of the cream during ripening.

With the semi-commercial churnings the ratios of the diacetyl contents of the cream to those of the corresponding butter were: minimum 1:0.142; maximum 1:0.709; and average 1:0.352. With acetylmethylcarbinol the ratios were 1:0.059; 1:0.683; and 1:0.217, respectively.

The diacetyl or acetylmethylcarbinol contents of the lots of butter in a series sometimes did not follow the same order as the contents of the lots of cream from which the butter was churned; the irregularities were of various types.

With the holding of butter 1 week at 36° to 40° F., 3 days at 60° F. plus 4 days at 36° to 40° F. or 1 month at 36° to 40° F., both increases and decreases in the diacetyl and acetylmethylcarbinol contents occurred. With each compound under each set of conditions the increases were more numerous than the decreases. After holding 3 days at 60° F. plus 4 days at 36° to 40° F., the diacetyl contents were higher in 75% of the comparisons, and the acetylmethylcarbinol contents were higher in 73% of the comparisons than in corresponding butter held in 1 week at 36° to 40° F.

Author's Summary

516. **Studies on Surface Taint Butter.** H. Wolochow and H. R. Thornton, Univ. Alberta, and E. G. Hood, Dom. Dept. of Agr., Ottawa. **IV. Distribution and Taxonomy of *Pseudomonas putrefaciens*.** Sci. Agr., *22,* No. 8: 461. 1942.

The literature on surface taint butter is summarized and indicates that *Pseudomonas putrefaciens* is widely distributed. It is a soil and water type. There is some confusion as to the taxonomy of this species. The authors state that this organism should be placed in the family Pseudomonadaceae, Tribe Pseudomonadeae.

V. The Growth of *Pseudomonas putrefaciens* in Butter. Sci. Agr., *22,* No. 9: 552. 1942.

There is much evidence that Gram-negative, rod-shaped, proteolytic bacteria which grow at low temperatures are involved in the production of surface taint butter. Large numbers of organisms must be present in the butter, however, if the organisms themselves are the cause of the defect, and the question is raised as to whether or not well worked commercial butters contain these large numbers.

In producing surface taint butter experimentally relatively large inocula had to be added to the cream or to the unworked butter. This also applied to the initiation of growth in tubes of litmus milk.

The numbers of bacteria in commercial surface taint butters were higher than in normal commercial butters but the surface taint producing organisms constituted only small minority populations in the defective samples. In commercial surface taint butters total counts ranged from less than 100 to more than 6,000,000. These numbers do not appear to be sufficiently large to account for surface taint defect on the basis of cell count.

The numbers of organisms in 5 inoculated-cream butters were determined at intervals up to 23 days, the butter being held at 10–15° C. In most of these cases the counts increased, the maximum numbers ranging from 10–17 million organisms per gram. Between 40 and 100 per cent of these were *Ps. putrefaciens.*

VI. Other Bacterial Species as Causal Agents. *Flavobacterium maloloris* (n. sp.). Sci. Agr., *22*, No. 10: 637. June, 1942.

Among 2500 random bacterial isolations there were 5 of a yellow-pigmented organism which produced surface taint in experimental butter and a sweaty-feet odor when grown in skim milk. The morphology and cultural characteristics of the organism are presented. It is a rod with rounded ends, occurs singly or in pairs, is Gram-negative, non-spore-forming and non-motile. On all media a yellow or yellow-orange color was produced. The organisms were highly proteolytic in skim milk and hydrolyzed starch. Glucose, sucrose, maltose or lactose did not appear to be utilized. The tentative name *Flavobacterium maloloris* is suggested for the species.

The ability of *Pseudomonas fluorescens* to produce surface taint was also investigated. Seven cultures of this species were used in 20 experimental butters some of which developed a surface taint or questionable surface taint 24 hours after churning. Within a further 24 hours these butters graded rancid. It appears that this organism is not a major cause of surface taint.
O.R.I.

517. Influence of Method of Separation on Mold Content of Cream. P. R. Elliker and W. H. Brown, Purdue Univ., Lafayette, Ind. Natl. Butter and Cheese Jour., *33*, No. 6: 10. June, 1942.

This experimental study was made to evaluate various methods of separating cream in terms of their effects upon the mold content and quality of the cream.

"Centrifugal separation resulted in lower mold content than did water-dilution and shallow pan separation when cream obtained by these methods was stored at 60° and 80° F. for four and seven day periods.

"When the fresh milk contained a comparatively high number of molds, centrifugal separation removed approximately 90% from both the cream and skim milk.

"Water-dilution and shallow pan separation tended to concentrate molds in the cream. This was apparently due to the effect of the fat globules which, as they rose, carried molds with them to the cream layer.

"Centrifugal separation with a clean centrifugal separator was superior to gravity separation both from standpoint of efficiency of separation and general quality of cream produced." W.V.P.

518. Propionate Salts as Mold Inhibitor. ANONYMOUS. Natl. Butter and Cheese Jour., *33*, No. 6: 16. June, 1942.

Propionate salts can be used to increase 2 or 3 times the mold-free life of butter and cheese. Parchment paper manufacturers make an impregnated paper but when butter is "wet-wrapped" the parchment is soaked in a solution containing 20 oz. of propionate salt per gallon of water. In cream and cottage cheese the salts may be added directly to the cheese at the rate of two to two and one-half ounces per 100 pounds of cheese.* Cheese such as Cheddar when sliced for distribution in stores may be immersed for 15 seconds in a solution of the inhibitor (proper concentration is not indicated) to increase the mold-free life of the cheese 3 to 4 times. W.V.P.

519. Advice on Neutralization. S. T. COULTER, Univ. Minn., Minneapolis. Am. Butter Rev., *3*, No. 12: 432–434. 1941.

Cream containing .17 to .18% acidity should be neutralized to reduce loss of fat in the buttermilk, to secure uniformity of taste, and to improve keeping quality. If starter is to be used acidity should be reduced to 0.1%; if not, to 0.12%. If acidity is higher pH is a better criterion by which to judge the degree of neutralization. For salted butter enough neutralizer should be added that the butter sera falls within the range of pH 6.6 to 7.0. If caustic soda is used it should be added in 5% solution, cold, and to the cream when it is no warmer than 80° F. Sodium sesquicarbonate has proved itself an excellent neutralizer, sodium bicarbonate is apt to give a soda taste, and limes, athough excellent, cause greater fat losses in the buttermilk. P.S.L.

CHEESE

520. Factors Affecting Mold Content and Quality of Cream. P. R. ELLIKER AND W. H. BROWN. Purdue Agr. Expt. Sta. Bul. 465. Jan., 1942.

The results obtained indicate that use of clean utensils and a storage temperature of 60° to 65° F. will insure satisfactorily low mold growth in cream over a storage period of 4 days. Temperatures as high as 70° F. may be employed over a 4-day period if utensils are kept scrupulously clean and milk or cream protected from contamination with dust and dirt. Delivery at least twice a week during colder months and three times a week during warmer months of the year is recommended. Where initial contamination of cream is high, excessive growth of molds may occur in as little as 2 days.

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* **Abstractor's Note:** Federal definitions require labeling the package when these salts are so used.

The method of separation of cream on the farm is an important factor (along with sanitation, time of storage, and temperature of storage) affecting mold content and quality of cream. Centrifugal separation removed a considerable portion of the mold growth from cream, but gravity separation tended to concentrate molds in the cream layer. Gravity separators also present problems in cooling and contamination of cream from water used in water dilution separation.

Twice-daily agitation of stored cream tends to retard mold growth and encourage development of yeasts. It is not recommended. On the basis of results obtained, it is recommended that fresh, cooled cream be added to stored cream by layering it over the surface of the older cream. A relationship was shown between low fat content and high mold and also small size shipments and high mold content in typical commercial cream samples. The factors entering into this relationship are discussed briefly. Pure cultures of *Oospora lactis* developed best when the culture medium was adjusted to pH values of 5.0 to 8.0. Definite inhibition of growth occurred at pH values below 5.0. P.R.E.

521. Conditions Necessary for Maintaining Quality of Year-Old Vacuum-canned Cheddar Cheese. N. S. GOLDING, Wash. Agr. Expt. Sta., Pullman. Natl. Butter and Cheese Jour., *33*, No. 7: 10. July, 1942.

Measurements were made of the changes in vacuum or pressure and quality of American Cheddar cheese ripened in cans in cool storage for over a year and then held at temperatures to simulate conditions in store or household. Changes in vacuum or pressure were determined by measuring the vacuum on the outside end of the sealed can necessary to raise the lid. Actual vacuum or pressure was then calculated by referring to a similar measurement made on an unsealed can. Six makes of year-old cheese were used. Cans of cheese from each make were stored for 50 days and observed. The data show that high-quality, year-old, vacuum-canned American Cheddar cheese should be held at 52° F. or below to maintain quality. If held at about 70° F. gas is developed and quality deteriorates. Gas developed faster in the latter half of the 50-day storage period. W.V.P.

DISEASE

522. Physiologic and Metabolic Aspects of Acetonemia in Cattle. M. H. ROEPKE, Univ. Minn. Jour. Amer. Vet. Med. Assn., *100*, No. 782: 411. May, 1942.

The article is a summary of recent findings as to the pathology and physiology relating to acetonemia together with a discussion of the value and limitations of the Ross test, the usual diagnostic test using nitroprusside as the diagnostic agent.

Pathologically, the severe form of the disease usually occurs up to six weeks post-partum in high-producing cows during the winter months. Severity of symptoms usually follows the degree of hypoglycemia, but does not parallel the blood and urine ketone concentrations. Fatty livers are a frequent post-mortem finding in severe cases.

Most conditions causing a cow to go off feed will produce a secondary ketosis and a positive Ross test, while a primary ketosis with clinical symptoms, blood ketone values above 20 mg. per cent, and urine ketones above 100 mg. per cent, is the exception and occurs when blood sugar values are one-half normal or less. Diagnosis is more accurate when the urine for the test is diluted 1 : 10 and still gives a strong reaction.

Fundamental causes of acetonemia may possibly be lowered liver function, inadequate pituitary stimulation, or lowered carbohydrate intake on winter rations. S.A.F.

523. The Value of Intravenous Calcium Chloride and Glucose in Uterine Inertia of Cows. H. C. SMITH, Stillwater, Okla. Jour. Amer. Vet. Med. Assn., *100,* No. 780: 232. March, 1942.

Where not contra-indicated, 50–250 cc. intravenously of a solution containing 20 per cent calcium chloride and 50 per cent dextrose in physiological saline eased and shortened delivery in cows with a history of protracted labor during previous calvings. There were no cases of milk fever or retained placentae and cows and calves both appeared stronger than is usually the case. S.A.F.

524. Sulfapyridine in the Treatment of Calf Pneumonia. W. T. S. THORP, J. F. SHIGLEY, AND A. K. ANDERSON, State College, Pa. Jour. Amer. Vet. Med. Assn., *100,* No. 780: 225. March, 1942.

Twenty-four calves, eighteen of which had symptoms of acute bronchopneumonia, were treated with sulfapyridine. Sixteen of the eighteen recovered. Best results were obtained by a high initial dosage and subsequent daily reduction of 0.02 gms. per lb. body weight. There was a significant temperature drop within 24 hours. Recommended initial dosages are 0.05 gms. per lb. body weight for calves up to 70 lbs.; 0.06 gms. per lb. body weight for 70 to 100 lb. calves; and 0.07 gms. per lb. body weight for calves weighing over 100 lbs. divided into three daily doses. Very young calves (less than 2 weeks old) are more susceptible to the drug and should receive smaller doses.

Blood studies on six normal and two pneumonic calves indicate a maximum blood level of 10 mgs. % with an average of 5 to 8 mgs. % is optimum for calves over two weeks old and an average of from 5 to 6 mgs. per cent for younger calves. Dosage is not proportional to age or body weight.

S.A.F.

525. Further Studies on the Significance of Suspicious Agglutination Reactions for Bang's Disease. C. P. Fitch, W. L. Boyd, Margaret D. Kelly, and Lucille M. Bishop, Minn. Agr. Expt. Sta., Jour. Amer. Vet. Med. Assn., *100*, No. 778: 23. Jan., 1942.

Results of twelve years of study on 136 animals are reported. Bacteriological plate cultures and guinea pig inoculations were made at regular intervals of milk, colostrum, placentas and vaginal discharges for the presence of *Br. abortus*. Cultures were also made of various organs of aborted fetuses and of calves which died shortly after birth. Aggultination titre of the blood and milk was determined regularly.

Group I consisted of 39 animals with a 1:25 to 1:50 titre throughout the period except for two animals which later became reactors and shed the organism. Two of 34 negative control animals housed with them became reactors and shed *Br. abortus*. All were removed from the group when their titre reached 1:100.

Group II consisted of a separately housed group of 97 animals entering the herd with titres incomplete at 1:100 or higher. Titres of this group varied from suspicious to positive or lowered to suspicious and remained. *Br. abortus* was isolated from nine of these animals.

It is suggested that, depending on herd history, animals with suspicious reactions may be sufficiently dangerous to warrant their removal from the herd. S.A.F.

526. A Further Note on the Incidence of *Trichomonas fetus* in Slaughtered Cattle from a Wisconsin Abattoir. B. B. Morgan and W. Wisnicky, Univ. Wisc. Jour. Amer. Vet. Med. Assn., *100*, No. 783: 471. June, 1942.

Trichomonas fetus was isolated culturally from two of 997 pregnant uteri, one with a normal, seven months fetus and one with a three months partially macerated fetus. Of 580 nonpregnant uteri, 463 were involuted, 100 had pyometra, 11 had retained placentae and 6 were recent parturitions. Thirteen cases of trichomonad infection were found in the pyometras. No cases were found in 211 bulls examined. S.A.F.

527. Studies in Ketosis in Dairy Cattle. IV. The Effect of Glucose Therapy and Pasture Feeding in Cases of Clinical Ketosis. J. C. Shaw, R. C. Powell, Jr., and G. C. White, Storrs Agr. Expt. Sta. Jour. Amer. Vet. Med. Assn., *100*, No. 783: 473. June, 1942.

Case histories of four clinical cases of Acetonemia together with blood and urine studies of ketone bodies, blood sugar and lactic acid levels during therapy are presented. Frequent administration of considerable quantities of glucose by various routes tended to keep ketones near normal, but did not consistently have a permanent effect. One case recovered when given access to pasture, but this was not consistently true. S.A.F.

528. The Treatment of Chronic Streptococcic Mastitis with Various Bactericidal Agents.

Ralph B. Little, Rockefeller Inst. Med. Res., Princeton, N. J. Internatl. Assoc. Milk Dealers Assoc. Bul. *34*, No. 16: 345. 1942.

Seldom does *Streptococcus agalactiae*, the most common cause of mastitis, appear in an acute form. Although usually a cause of the chronic form *Streptococcus dysgalactiae*, *Streptococcus uberis* and others may be responsible for acute forms. A detailed description of methods and equipment used for administering bactericidal agents is given. The acridine derivatives, Entozon, acriflavine, and also trypaflavin were administered by infusion. When an oil solution is injected via the teat canal directly into the milk cistern, advantages in large scale work are smaller volumes of solution, less equipment required and simplification of instrument sterilization. Gramicidin or tyrothricin and Novoxil have been used.

It is emphasized that chemotherapy is a surgical procedure and should be conducted by a veterinarian lest great damage to the udder result from careless technique. A conservative figure for cures in an average herd by means of udder chemotherapy might be below 50% with some herds going as high as 80–90% cures. Mild cases offer the best chance of success and chemotherapy should not be used on acute cases. Injection of tyrothricin and Novoxil may safely be made during the dry period to save on material. Of the bactericidal agents effective against *Streptococcus agalactiae* the author has found the alcoholic solution of gramicidin in sterile water and mineral oil with the dose properly adjusted, was the least irritating and most effective. E.F.G.

529. Studies on the Allergic and Antigenic Activity of Sonic Filtrate of Brucella Abortus.

E. L. Stubbs and I. Live, Univ. Pa. Amer. Jour. Vet. Res. *3*, No. 7: 146. April, 1942.

A brief review is made of the literature as regards attempts to produce allergic skin reactions to establish *Brucella* infections. The sonic filtrate was a 72-hour culture of *Br. abortus* concentrated, washed, suspended in saline, exposed to vibrations of audible frequency at 9,000 cycles per second for one to two hours and filtered. Groups of rabbits negative to the agglutination test in dilutions of 1:5 were sensitized by injecting live cultures of *Br. abortus* intravenously and intraperitoneally; phenolized cultures intravenously, and heated but not killed cultures intravenously. Guinea pigs were sensitized by intraperitoneal injections of live cultures. Reaction to the skin test at 48 and 85 days was nearly 100% in those receiving live cultures and decreased with the degree of attenuation. Intensity of reaction varied with the amount of protein in the injected filtrate. All sensitized animals were positive to agglutination, precipitation, and opsonocytophagic tests. One group of rabbits, sensitized by intracutaneous injections of sonic filtrate, did not react to subsequent skin tests but did react to the agglutination tests. Agglutination titre and the length of time before it disappeared were directly proportional to the amount of protein in the sensitizing injection of sonic filtrate. S.A.F.

FEEDS AND FEEDING

530. Experiments with Annual Crops and Permanent Pastures to Provide Grazing for Dairy Cows in the Sandhill Region of the Southeast. E. W. Faires, J. R. Dawson, J. P. LaMaster, and G. H. Wise. U. S. Dept. Agr. Tech. Bull., *805*. Nov., 1941.

The experiments reported in this bulletin were conducted at the Sandhill Expt. Sta., Columbia, S. C., to determine the extent to which annual crops and permanent pastures could be used to provide grazing for dairy cattle on the light soils of the sandhill region of the Carolinas and Georgia, and to ascertain also the relative economy of these two systems of providing nutrients for milk production. The experiments extended over a five-year period (1933–37) and utilized eight 2-acre grazing plots for the annual crops and a 6-acre pasture for the permanent pasture experiment. Annual crops selected were (1) for winter and early spring grazing, a combination of oats, barley, rye, and vetch, and (2) soybeans and pearl millet for summer and early fall. Other crops given a trial were (1) corn interplanted with velvet beans for fall, (2) crimson clover and Italian rye grass for spring, and (3) pearl millet alone for summer. Seeding mixtures for the permanent pasture were (1) for lowland pasture, common lespedeza, carpet grass, and white Dutch clover; (2) for upland pasture, common lespedeza and Bermuda grass. Dallis grass and hop clover were also used in the lowland.

The permanent pasture, while rather slow in forming a satisfactory sod and with a rather low carrying capacity, furnished nutrients much more cheaply than did any of the annual crops, singly or in combination. It is felt that the returns from the annual crops would have been greater if they had been made into silage instead of being grazed. Least costly annual crop was pearl millet, most costly the combination of rye grass and crimson clover, at $11.87 and $65.89 respectively per ton of alfalfa hay equivalent. On the same basis the permanent pasture cost $8.20 per ton. J.G.A.

531. Feeding and Management of Dairy Calves. C. H. Staples and D. M. Seath. La. Agr. Expt. Sta. Bull., *342*. March, 1942.

Suggestions on the feeding and management of dairy calves and yearling heifers based on records of feed cost and growth of 196 Jersey and Holstein calves over a period of eight years (1928–1935) at L.S.U. J.G.A.

532. Dairy Farm Management and Costs in Pennsylvania. W. L. BARR. Pa. Agr. Expt. Sta. Bul., *421*. Feb., 1942.

An analysis of 206 farm records shows that it cost an average of $2.26 to produce and market a hundred pounds of milk from 1938 to 1940, and averaged $2.13, $2.34, and $2.32 for 1938, 1939, and 1940, respectively. Feed and bedding costs averaged about 50%, man labor 30%, and other costs 20% of the gross cost of producing a hundredweight of milk. Returns per hour of labor from the dairy enterprise averaged 35¢ in 1938, 23¢ in 1939 and 29¢ in 1940. Returns to the operators of these farms as measured by labor income were $594 in 1938, $560 in 1939, and $755 in 1940. The important factors responsible for variation in costs and returns were size of business, labor efficiency, and rate of productions. Author's Abstract

533. Profitable Permanent Pastures for Dairy Cattle. D. M. SEATH. La. Agr. Expt. Sta. Bull., *341*. March, 1942.

A description of fertilizer experiments with pastures during 1937–40 inclusive in Claiborne, Lincoln, and Washington parishes, La. Reseeding, frequent mowing, and controlled grazing tended to increase the productivity of the pastures from year to year. Annual fertilization accelerated this improvement. Fertilized pastures (350 pounds 4–12–4 in the fall, 50 pounds $NaNO_3$ in May) averaged 58% more cow days of grazing, 64% more milk, and 44% more returns over cost of grain fed and fertilizer used than did unfertilized pastures. Annual returns showed an advantage of $16.05 per acre for the fertilized pastures after deducting feed and fertilizer costs. Yields of air-dry hay from protected areas in the pastures showed a 31% advantage for the fertilized areas and a 24% advantage in phosphorus content of the fertilized hay. Basic slag applied to the previously unfertilized pastures in the fifth year showed a somewhat greater response than was evident that same year from the previously fertilized pastures. Recommendations are given for a definite pasture program based on these results. J.G.A

534. Factors Affecting Profits from Dairy Herds. D. M. SEATH AND E. W. NEASHAM. La. Agr. Expt. Sta. Bull., *338*. Feb., 1942.

A study of D.H.I.A. records in Louisiana during 1938–40 inclusive, involving 32 herds in '38, 33 in '39, and 25 in '40. Although it cost more to feed them, high yielding cows returned $2.40 more per $1.00 of increased feed cost than did the low producers. Grain feeding at the rate of 1 pound for each 3.0 to 3.4 pounds of milk proved the most profitable. March to June, inclusive, were the high months both in production per cow and in average return over feed cost. Of two herds of comparable breeding, the one provided with better roughage over a two-year period, showed an increase of a gallon of milk per cow daily and $71.04 greater return over feed cost per cow yearly. Recommendations based on the findings are given relative to breeding, culling, record keeping, and pasture production.

J.G.A.

535. The Effect of Feeding Some Fat-Soluble Dyes to Milking Cows upon the Color of the Milk Fat.

C. F. HUFFMAN AND C. W. DUNCAN, Mich. Agr. Expt. Sta. Quarterly Bull., *24*, No. 1: 54–55. Aug., 1941.

In studying the effect of different fats on milk and fat production the authors were interested in following food fat through the digestive tract and into the milk. For this purpose five different fat-soluble dyes were fed, viz.: Sudan III, Sudan IV, Brilliant Green, Perfect Purple, and Nigrosine Black. The dyes were mixed with 0.5 pound of crude soybean oil with the exception of the Sudan IV which was mixed with solvent-extracted soybean oil meal. The dosage was 15 grams of dye all fed in one feed, except in case of Nigrosine Black where the dose was 45 grams.

It is apparent that milk fat is readily stained by feeding these dyes. Earliest appearance of the dye in the milk fat was in the case of either Sudan III or Sudan IV, where it appeared in the next milking 12 hours later, and persisted for 144 hours. Some of the dyes were evidently altered in their passage through the cows' systems; for example, Perfect Purple produced a green-colored butterfat, and Nigrosine Black a pink butterfat Either Sudan III or Sudan IV appears to be preferable for this purpose to the other dyes used.

J.G.A.

536. A Cobalt Deficiency Observed in Some Michigan Dairy Cattle.

A. C. BALTZER, B. J. KILLHAM, C. W. DUNCAN, AND C. F. HUFFMAN, Mich. Agr. Expt. Sta. Quarterly Bull., *24*, No. 1: 68–70. Aug., 1941.

In a study of the disease of dairy cattle colloquially known as "Grand Traverse or Lake Shore Disease" and recognized in Michigan for many years, clinical evidence was obtained which indicated that the condition is essentially due to cobalt deficiency. The blood of affected animals showed a very low concentration of hemoglobin; evidence of ketosis or phosphorus deficiency was lacking. The animals responded very quickly in appetite and vigor to the feeding of a cobalt supplement (either chloride or sulphate) although hemoglobin regeneration was slow. Preliminary investigation showed that the cobalt content of hay grown on affected farms is much lower than hay grown on farms in unaffected areas.

J.G.A.

537. **The Effect of a High Vitamin A Intake on the Blood and Milk Carotene of Holstein and Guernsey Cows.** H. J. DEUEL, JR., L. F. HALLMAN, C. JOHNSTON AND F. MATTSON, Dept. Biochem., Univ. Southern California, Los Angeles. Jour. Nutr., *23*, No. 6: 567. June, 1942.

The cause of the depression of carotene in milk upon the addition of vitamin A to the ration was investigated, using 25 Guernsey cows in one experiment and 26 Holsteins in a second experiment. In each experiment the cows were divided into three groups: group one received 30 ml. of shark liver oil which furnished 700,000 I.U. of vitamin A per cow per day; a second group received an equal amount of vitamin A as a concentrate assaying 545,000 I.U. per gram; a third group were used as controls. In the second experiment, the group of Holstein cows which received the vitamin A concentrate were fed different levels of vitamin A.

The decrease in milk carotene appeared to be caused by the vitamin A *per se.* A constant decrease in blood carotene was also observed when the milk carotene was lowered. Approximately 4 weeks were required for the maximum lowering in carotene to develop while 7 to 10 weeks were required after the cessation of the vitamin A supplement for the return of carotene secretion to normal.

The average increase in vitamin A was 47 units per gram of butterfat for 1,000,000 I.U. The maximum level of vitamin A observed was 331 I.U. per gram. About 3 per cent of the ingested vitamin A was recovered in the milk. C.F.H.

ICE CREAM

538. **Substituting for Coconut Fat in Dipping Chocolate.** J. J. SHEURING AND P. H. TRACY, Univ. Ill., Urbana. Ice Cream Field, *39*, No. 6: 28. June, 1942.

Difficulty in obtaining coconut oil as a substitute for cocoa butter in chocolate coatings, extensively used in the ice cream industry, prompted the authors to try other fats for this purpose. They give the following as characteristics of good chocolate coating:

(1) Pleasant flavor without waxy after effect in the mouth, (2) firm body when cooled but not brittle enough to fall off ice cream bars while being consumed, (3) resistant to melting at room temperatures, (4) rapid solidification when cooled on ice cream bars, (5) resistant to thickening when moisture is absorbed during dipping, (6) good dipping coverage at 100° to 110° F., and (7) uniform chocolate color.

Experiments were conducted in which soybean oil, hydrogenated soybean oil, coso oil, beef fat, hydrogenated cottonseed oil and corn oil were used as substitutes for coconut oil. Samples were prepared by diluting coating with 8 to 25% of domestic oils and fats, and compared with a standard milk chocolate coating as a control. "The results indicate that 8 to 15% of low melting point fats and 10 to 25% of high melting point fats can be used successfully as substitutes for coconut oil. The best product was made by using a combination of 5% added hydrogenated soybean oil and 5% soybean oil."

They also state that using 10% cocoa, 40% powdered sugar, 35% hydrogenated soybean oil and 15% soybean oil could be used as a satisfactory coating for emergency use although it was not as desirable as one prepared from chocolate liquor. W.C.C.

539. The Case for the Soda Fountain. L. G. Blessing, Pres. of Bastian Blessing Co. Ice Cream Field, *39*, No. 5: 14. May, 1942.

The author quotes Joseph J. Hammer, attorney for the New York State Pharmaceutical Association, as stating that the soda fountain is an undesirable intruder in the retail pharmacy and that the fountain-luncheonette is "on the way out." Statements and figures are then presented by the author to show that instead, the soda fountain is "on the way up."

According to the author, reports of the U. S. Department of Commerce show that in the United States in 1929 there were 34,844 drug stores with fountains and 23,414 without fountains. During the period 1929 to 1933 the number of drug stores with fountains increased to 38,448, while 3,455 stores without fountains either failed or voluntarily discontinued business. During the entire period 1929 to 1939 the reports show an increase of 13.2 per cent in the number of drug stores with fountains and a decrease of 21.2 per cent in the number of stores without fountains.

The author concludes that during prosperity and depression a soda fountain makes a druggist's investment safer and more profitable than it would be without one. W.C.C.

540. Testing Ice Cream for Fat by the Pennsylvania Method. W. D. Swope, Pa. State College, State College, Pa. Ice Cream Field, *39*, No. 5: 16. May, 1942.

According to the author approximately 3,000 carefully controlled tests were made during a ten-year period to find reagents which would prevent carbonization of added sugar, give a clear fat column, and upon centrifuging, completely liberate fat from dairy products containing chocolate or added sugar. The approved Mojonnier method was used as a comparison for all of these tests.

The Pennsylvania method has been developed as a result of these tests. It makes use of regular Babcock test equipment and reagents that are readily obtainable from chemical supply houses. The reagents are: (1) ammonium hydroxide, 28 to 29 per cent NH_3, (2) normal butyl alcohol, b. p. 117° C., (3) diluted sulphuric acid, specific gravity approximately 1.72 to 1.74.

Details of preparing the samples and the procedure to follow in testing are given.

Tabulations of results obtained by this method show them to be in very good agreement with the approved Mojonnier method. It is concluded that the Pennsylvania method is well adapted for determining the percentage of fat in ice-cream mix as well as ice cream of all flavors. It is stated further that comparable results can be obtained by different operators.

Information regarding the testing of other dairy products by this method may be found in Pennsylvania Agricultural Experiment Station Bulletin 412. W.C.C.

541. 1941 Ice Cream Sales Hit All-Time High. O'NEAL M. JOHNSON, Statistical and Accounting Bur. Ice Cream Trade Jour., *38,* No. 5: 19. May, 1942.

Ice cream sales hit a new high in 1941. The percentage of gain, based on reports from 773 manufacturers, was 18.27%. Increases by period were January–April, 21.05%, May–August, 17.22%, and September–December, 20.75%. December, 1941, was the fifteenth consecutive month in which the nation showed increased ice cream sales over the corresponding month of the previous year. By sections, the North Atlantic Area had an increase of 18.62% for the year, Central East Area, 17.94%, Middlewest Area, 15.84%, Southern Area, 26.29%, and the Western Area an increase of 9.97%. Arizona and New Mexico were the only states not showing an increase. Part of the Southern Area increase was due to a concentration of army camps in that region. Temperatures for 1941 were exceptionally favorable except during February and March. W.H.M.

542. Sugar and Sherbets in Wartime. R. I. MASUROVSKY, Res. Ed. Ice Cream Trade Jour., *38,* No. 5: 32. 1942.

Sherbets are more important to the dairy industry now than they were in the first World War. Many manufacturers are decreasing the sugar content of their ice cream mix one or two per cent and the sherbet mix may be done likewise without serious ill effects. Corn sweetness or invert sugar should be used in combination with cane sugar in sherbets and the numbers of flavors offered should be reduced to two or three. W.H.M.

543. A Neglected Phase of Frozen Desserts Sanitation. F. W. FABIAN, Res. Prof. of Bact., Mich. State Col., East Lansing, Mich. Jour. Milk Technol., *5,* No. 2: 106. Mar.–Apr., 1942.

Next to milk, frozen desserts are the important cause of epidemics.

The ingredients, such as coloring material, flavoring extracts, fruits and nuts, going into the mix after pasteurization should be given a germicidal treatment equivalent to pasteurization. Treatments for so doing are discussed. L.H.B.

MILK

544. How Methods and Promptness of Cooling Affects the Quality of Milk. A. J. Gelpi, C. S. McCleskey and D. M. Seath. La. State Univ. Bull., *344*. 1942.

Comparisons were made between milk cooled in 10-gallon cans in a motor-stirred water tank (33°–40° F.) that cooled on a surface tubular cooler followed by holding in milk cans in 33° to 40° F. water, and that cooled on a conical cooler followed by holding in milk cans in 33° to 40° F. water.

Slightly higher bacterial counts were obtained in the milk cooled in cans. Delayed cooling (2 hours) did not materially affect the quality of the milk when it was cooled over a tubular or conical cooler. When the subsequent cooling was done in the milk can a significant increase in count after holding 12 hours resulted from the delayed cooling.

There was no significant difference in the flavor of the milks cooled by the three different methods.

Can cooling in an over-loaded cooling tank was the least effective cooling method studied. P.H.T.

545. Supply Responses in Milk Production in Southeastern Minnesota. E. G. Strand and E. Hole. U. S. Dept. Agr.,Technical Bull., *798*. 1941.

The primary purpose of this bulletin was the determination some years in advance of a long-time supply schedule for butter fat in an area composed of five counties in southeastern Minnesota. Past trends in production were analyzed for the area as a whole and estimates were formulated of the future production of the area in three possible future price situations: (A) a continuation of 1935 normal prices; (B) a 20% increase in butterfat prices; and (C) a 20% decrease in butterfat prices.

Briefly the changes that occurred in the area from 1927 to 1938 were as follows: butter-fat production apparently increased about 12%, due to a corresponding increase in number of cows. Feed producing capacity was increased 13%. Due to drouth and the A.A.A. program hog production was drastically reduced in 1934, but later the trend was distinctly upward again. Since 1935 production of beef cattle has tended to increase. Poultry and sheep have tended to increase during the entire period. Reduction in number of horses has continued.

Assuming continuation of the normalized prices prevailing in this area in 1935 (situation A), the normal production of butterfat in the area will be 9% higher in 1945 than it was in 1935.

Assuming an increase in the relative price of butterfat (situation B), some transfer of resources from hogs and poultry to dairy cattle may be expected. However, dairying is not sufficiently superior on many farms to warrant more than a moderate increase in it at the expense of other enterprises.

Assuming a relative decline in butterfat prices (situation C) its production is expected to be slightly less than the normal output in 1935. This contraction would be relatively larger than the estimated expansion under situation A.

Comparisons with areas in New England and Wisconsin indicate that responses in dairy production are influenced by alternative enterprises and by changes within the dairy enterprise itself. J.G.A.

546. Technical Control of High-Temperature, Short-Time Equipment. D. M. ROGER, Borden's Farm Products, Brooklyn, N. Y. Internatl. Assoc. Milk Dealers, Assoc. Bul., *34,* No. 17 : 376. 1942.

In checking the holding time of a STHT unit it is recommended that a cross check be made by two methods, one the conductivity method and the other a calculated holding time based upon the capacity of the holding tube in quarts per second. The calculated holding time will fall upon a straight line graph and the nearer the conductivity results fall on a straight line parallel to it the more reliable they are. Deviations from a straight line by more than .5 seconds should be regarded with suspicion. E.F.G.

547. Experimental Work on Deaeration of Milk. P. F. SHARP, D. B. HAND AND E. S. GUTHRIE, Cornell Univ., Ithaca, N. Y. Internatl. Assoc. Milk Dealers, Assoc. Bul., *34,* No. 17 : 365. 1942.

The defect, oxidized flavor, in milk can in general be prevented by deaeration of the milk and by bottom up filling to prevent reincorporation of air so that the final product contains below 0.4 to 0.5 mgs. of oxygen per liter.

Data on summer milk as received at New York City pasteurizing plants indicates 13 mgs. of reduced and 4.5 mgs. of dehydro ascorbic acid and 9.5 mgs. of oxygen per liter. Oxidation of the ascorbic acid is hastened by copper contamination in country milk plants. Morning's milk received at the country plants contained 20.4 mgs. of reduced and 0.5 mgs. of dehydro ascorbic acid per liter and night's milk 18.8 mgs. and 1.5 mgs. respectively. Hand milking introduced more oxygen than machine milking, the averages being 5.8 and 4.7 mgs. respectively.

Streptococcus lactis activity sufficient to produce 0.05% developed acidity was necessary to retard oxidation of reduced ascorbic acid. It is thought by the authors that retardation of oxidized flavor by bacteria may be due to factors other than removal of oxygen. Extensive tabular data support the above points. E.F.G.

548. First Annual Producers' Meeting—Introductory Remarks. R. C. ROUCHE, Telling-Belle Vernon Co., Cleveland, O. Internatl. Assoc. Milk Dealers, Assoc. Bul., *34,* No. 19 : 401. May, 1942.

A sample producers meeting is reproduced. These meetings are for the purpose of discussing producers timely subjects. In this sample meeting an address is given by J. C. Nisbet, Extension Director of the Am. Jersey Cattle Club on the subject "Efficient Milk Production" and an Ohio 4-H Calf Club demonstration on the care and handling of a milking machine. Specific directions and a suggested hour to hour program are given for such a producers meeting. E.F.G.

549. Are Standard Costs Practical in Our Industry? W. T. GRANLUND, White Bros. Milk Co., Inc., No. Quincy, Mass. Internatl. Assoc. Milk Dealers, Assoc. Bull., *34,* No. 18: 395. 1942.

Since standard costs furnish performance goals the effect of the use of this system upon efficiency is important. The standard cost system is divided into (1) City Plant, (2) Containers, (3) Selling and Delivering. The above items are further broken down. Each month a variance sheet shows the difference between actual cost and that of the standard cost system.

E.F.G.

550. Can Operating Costs be Reduced by the Use of Multiple Containers? CHARLES A. ARMITAGE, United Farmers Cooperative Creamery Assoc., Inc., Boston, Mass. Internatl. Assoc. Milk Dealers, Assoc. Bul., *34,* No. 18: 387. 1942.

Summing up the plant savings which can be made in putting out milk in two-quart rather than one-quart bottles the conclusion is reached that this amounts to about one-quarter of a cent per quart or a total saving of one-half cent on the two-quart container. The general statement is made that the saving in delivery should be much greater than in plant operation but no figures are given. E.F.G.

551. Methods for Compensating Routemen when Routes are Split. SAMUEL GAILEY, Supplee-Wills-Jones Milk Co., Philadelphia, Pa. Internatl. Assoc. Milk Dealers, Assoc. Bul., *34,* No. 18: 383. 1942.

An equitable formula in compensating a routeman when his route is split should give consideration to the following factors: 1. Present method of compensation—A basic weekly wage will not be reduced but commissions will be. 2. Stabilization of the earnings of the routeman. Compensating for commissions until these can be built up again. 3. Reasonable cost to the company. So that new business will not be too costly.

The following formula has been included in union agreements and has been found practicable from the standpoint of labor and management. "When a route is split, the routeman shall receive in addition to the amount actually earned on such route as split for the first week after the split, an amount equal to the weekly loss in commissions due to the split. Thereafter this amount shall be progressively reduced each succeeding week by an amount obtained by dividing such weekly loss in commissions due to the split by one-half of the number of quarts lost due to the split." Such progressive weekly reduction is to continue until the amount equal to the weekly loss due to commissions due to the split has been exhausted. The "weekly loss in commissions due to the split" is the weekly commission on the average weekly sales to the customers taken from the route for four weeks prior to the split; and the number of quarts lost due to the split "is the average daily number of quarts delivered to such customers during such four-week period." The above is based upon keeping the routeman's compensation level by the acquisition of two quarts of new business per week. E.F.G.

552. Effect of Water Bacteria on Quality of Dairy Products. W. B. SARLES Univ. Wis., Madison. Natl. Butter and Cheese Jour., *33*, No. 7: 14. July, 1942.

Bacteria from air, soil and sewage may contaminate water; for use in a dairy plant water must be free of sewage pollution and should contain very few bacteria of air or soil origin. Water from cooling tanks or water used for rinsing equipment may get into milk; washing buttermilk from butter illustrates how water may contaminate a manufactured product. Waterborne contamination may cause May apple flavor, rancid, putrid, cheesy, or unclean flavors and gassiness in milk or cream held at 32° to 50° F. for more than one day; similar defects may be expected in bulk evaporated milk stored at 32° to 45° F. for several days. Water bacteria in butter may cause May apple flavor, rancid, potato, cheesy, skunk and putrid flavors; churns or equipment may be carriers of such bacteria. Water bacteria may cause gas or bitter flavors in cheese. The bacteriological quality of the water supply should be determined frequently; pasteurization or chlorine disinfection should be used when necessary for water that may contaminate the equipment or products. W.V.P.

553. Detecting Bloody Milk. H. J. BRUECKNER, Cornell Univ., Ithaca, N. Y. Am. Milk Rev., *3*, No. 11: 260–261. 1941.

Bloody milk may be detected by allowing the first few streams to flow over a piece of black bakelite, on which it appears to have a pink color. If a half pint is held 12 hours the cream layer may show the presence of blood. Or the first few streams of suspected milk may be placed on filter paper, a few grains of powdered benzidine added, followed by a few drops of glacial acetic acid, and 4 to 6 drops of hydrogen peroxide. A positive test is indicated by a blue to green discoloration. P.S.L.

554. Flexible Prices for Milk. LELAND SPENCER, Cornell Univ., Ithaca, N. Y. Am. Milk Rev., *4*, No. 1: 10–11. 1942.

Determination of a just price and delay in adjustment to changing conditions are two major difficulties in fixing milk prices. The latter difficulty may be solved by adoption of a formula for setting the price. A few objections to prices set by production costs are purchasing power of consumers and competition from other buyers of milk. Judgment must be exercised, depending upon the quotations upon which the formulae are based and unusual conditions such as drought. P.S.L.

555. Homogenization of Milk to Prevent Excessive Fat Rising. H. J. BRUECKNER, Cornell Univ., Ithaca, N. Y. Am. Milk Rev., *3,* No. 10: 235–236. 1941.

No advantage was found in using two-stage homogenization to prevent fat rising. A pressure of 3000 pounds was necessary with the single stage at pasteurizing temperature, and produced milk meeting the standard of the American Public Health Service. If milk below the upper hundred ml. portion tests 4%, that in the upper one hundred ml. layer must test no more than 4.2%, or 5% greater than the body of the milk, after 48 hours storage. P.S.L.

556. Factors in Price Determination. LELAND SPENCER, Cornell Univ., Ithaca, N. Y. Am. Milk Rev., *4,* No. 1: 16–17. 1942.

The Federal Marketing Agreement Act of 1937 set up parity of purchasing power as the major consideration in setting milk prices, although under certain conditions this consideration may be set aside entirely. Reliable statistical data as to demand, supply, and price levels, are essential for sound price making, and they must be utilized intelligently in forecasting conditions. •Each factor must be weighted to judge its relative value. Examples from the New York market are given to illustrate those changing factors that must be given consideration in arriving at as near just prices as possible. P.S.L.

557. Minnesota Babcock Test Reagent. S. T. COULTER, Univ. Minn., Minneapolis. Am. Butter Rev., *3,* No. 8: 282. 1941.

Minnesota reagent has proved satisfactory for use in schools where danger from acid is a factor, but is not advised in states not recognizing the test in the buying of milk and cream. It has proved very satisfactory for the testing of ice cream and condensed milks in laboratory control but has not been considered an official test. For buttermilk testing it has given results that may be duplicated. P.S.L.

558. Effective Can Washing. V. SCHWARTZKOPF, Lathrop-Paulson Co., Chicago, Ill. Am. Butter Rev., *4,* No. 1: 10–16. 1942.

A complete resume of results secured in several plants through use of Mikro San acid for can washing is given. A cleaner and more nearly sterile can was secured through its use due to atomization of the cleaner, use of clean wash water, removal of solids from wash water, high temperature wash water, longer exposure to heat, rapid drying, prevention of stone formation, and keeping the can on the neutral or acid side. The cleaner reduced greatly the thermophylic, thermoduric, lipolytic, and proteolytic types of bacteria. It reduced steam costs 30 to 60%, feed water 50 to 75%, detergent 70%, and required less labor. Other benefits from the use of a cleaner of acid reaction were listed. P.S.L.

559. Symposium: Opportunities for Reducing Operating Cost. (A) Butterfat Cost. F. Bruce Baldwin, Jr., Baldwin Dairies, Inc., Philadelphia, Pa. Internatl. Assoc. Milk Dealers, Assoc. Bul., *34*, No. 15: 325–330. Feb., 1942.

The butterfat method of accounting for milk plant losses is suggested as the most accurate. Losses beyond 2% are considered excessive. By a record of weights of milk and cream used by each department, drip sampling on flow lines and by vat sampling it is possible to locate the department in which losses occur. Affecting the accuracy of a fat accounting system are (1) accuracies of counting by plant men, (2) accuracy of tests, (3) suitable conversion factors. Conversion factors appropriate to the temperatures actually used should be employed. E.F.G.

560. Symposium: Opportunities for Reducing Operating Cost. (B) Plant Cost. J. F. Malone, Borden-Wieland, Chicago, Ill. Internatl. Assoc. Milk Dealers, Assoc. Bul., *34*, No. 15: 331–336. Feb. 1942.

More than 90% of all milk plant operating costs are made up of the following: labor, property expense, caps, cartons, parchment, repairs to buildings and equipment, product waste, fuel, electricity, bottle breakage, washing powder, and water. Labor represents about 50% of all plant costs and should be broken down by operations so that excessive amounts of labor or variations in requirements will be evident by noting the labor requirements per unit of product. Accurate figures on usage for various supplies will disclose when current consumption is excessive. E.F.G.

561. Public Health Aspects of Reciprocal Inspection of Milk and Cream Supplies. George W. Grim, Ardmore, Pa. Internatl. Assoc. Milk Dealers, Assoc. Bul., *34*, No. 14: 319–322. Feb., 1942.

The primary responsibility for enforcing requirements "looking to the promotion of quality sanitation and decency in practices prevailing upon dairy farms producing milk afterwards to be pasteurized rightfully belongs to the buyer of milk, not to the Health Department. The Health Department of the state or city should be expected to assume the most active official supervision which at all times should be secondary to the control and supervision of the buyer." The Health Department discharges its major responsibility which is protection of health when it assures supervision of effective pasteurization. Needless duplication of inspection of dairy farms could be eliminated by fairly uniform rules and regulations together with reciprocal arrangements. The details of regulations with reference to quality should come from buyer suggestions. E.F.G.

562. Reciprocity Inspection of Milk and Cream Supplies. TOM G. STITTS, U. S. Dept. Agr., Washington, D. C. Internatl. Assoc. Milk Dealers, Assoc. Bul., *34,* No. 14: 313–318. Feb., 1942.

Reciprocity in general is "The full acceptance by each of two or more milk inspection authorities of the inspection and certification of each other." Four types of reciprocity are: 1. Between plants in the same milkshed; 2. Between cities receiving milk from the same milkshed; 3. Between separate milksheds; 4. Between widely separated milksheds.

Although the first two are clearly advisable, real difference of opinion exists with regard to the last two. Technical, economic and political factors are involved. It is contended that although the lack of reciprocity has been to raise milk and cream prices this rise has been negligible. It is stated that reciprocity between milksheds would discriminate against local milk dealers and favor the development of national and regional chain-dealer organizations. A marked loss of bargaining power by local producer cooperatives would result in a trend to larger cooperatives. Changes will undoubtedly come involving simplification and standardization, but the desire of the public for fresh milk and their confidence in the inspection service under which it is produced are important considerations. E.F.G.

563. New Areas of Supply and Demand. J. L. DAVIES. Milk Indus., *24,* No. 10: 23. 1942.

Taking the total sales of milk in 1941, consumption in Great Britain as a whole was about twenty-five per cent higher than in the pre-war period. The increase was greatest in the North and while milk travelled South in large quantities before the war, the movement in 1941 was the other way. The greatest increase in consumption was by families with the lower incomes per head.

Following the great increase in consumption of milk, it has been necessary to draw supplies of liquid milk from the more remote parts of the country. A high proportion of this milk is produced on small farms where there are inadequate facilities for cooling and preparing the milk for the liquid market, resulting in difficulties through souring.

The most important change, perhaps, is the increase in the number of small producers since 1939. No less than 25,000 farms, mostly small, have placed their milk on the market for the first time. About half the total of 150,000 producers have less than twelve cows. Only about 25,000 farms are accredited. J.J.S.

564. Turn Off the Heat. ANONYMOUS. Natl. Butter and Cheese Jour., *33*, No. 5: 12. May, 1942.

It is essential for quality control and economy that milk and cream should be cooled promptly and to a low temperature. Common methods are described and a U. S. Geological Survey map of the United States is given showing well-water temperatures at 30 to 60 ft. depths. W.V.P.

PHYSIOLOGY

565. Clinical Observations on the Use of Equine Gonadotropin in the Mare and Cow. H. S. CAMERON, Univ. Calif. Jour. Amer. Vet. Med. Assn., *100*, No. 778: 60. Jan., 1942.

"Nine mares and 46 cows were treated with equine gonadotropin in an attempt to induce estrus where the cycle was dormant. All of the mares responded within ten days, whereas 25 cows failed to respond. Judging from the results, the hormone was highly effective in mares, considerably less effective in cows." S.A.F.

566. Increase in Weight of Gonads Following Injection of Single Male Rat Pituitary. R. P. REECE AND E. J. WEATHERBY, N. J. Agr. Expt. Sta. Proc. Soc. Expt. Biol. and Med., *49*, No. 2: 218. Feb., 1942.

Pituitary glands were collected from 80 sexually mature male rats and injected into eighty 24- to 26-day-old female rats. Injections were made subcutaneously twice daily for 4 days and the recipients killed 100 hours after the first injection and the ovaries weighed. For controls the ovaries of fifty-seven 28- to 30-day-old non-injected rats were weighed. Average ovarian weight of the control animals was 13.4 mg. whereas that of the injected animals was 54.8 mg. Sixteen similar pituitary glands were injected into 16 one-day-old male white Leghorn chicks and the testes weighed 100 hours after the first injection. Thirty chicks receiving no treatment served as controls. Average testicular weights of the control and of the injected chicks were 9.5 mg. and 15.4 mg. respectively. R.P.R.

567. Effect of Estrone on Lactogen Content in Pituitary and Blood of Male Rabbits. JOSEPH MEITES AND C. W. TURNER, Univ. Mo. Proc. Soc. Expt. Biol. and Med., *49*, No. 2: 190. Feb., 1942.

Three groups of male New Zealand White rabbits were injected with 3 different levels of estrone and a fourth group served as controls. Pituitary assays revealed that a considerable increase in lactogen content was obtained, although a dosage of 5000 I.U. of estrone produced a smaller increase than did either 500 or 1000 I.U. Assays of whole untreated rabbit blood showed that a definite increase in the release of lactogen into the blood was secured following estrone injection. R.P.R.

568. Role of Estrogen in the Stimulation of Mammary Lobule-Alveolar Growth by Progesteron and by the Mammogenic Lobule-Alveolar Growth Factor of the Anterior Pituitary. JOHN P. MIXNER AND CHARLES W. TURNER, Univ. Mo. Endocrinol., *30*, No. 2: 591. April, 1942.

The range of dosage which would best synergize with 1 mg. of progesterone in stimulating mammary lobule-alveolar growth in the ovariectomized virgin mouse was found to be quite wide; however, a dosage of 100 I.U. was found to be optimal. When progesterone was injected alone approximately 6 times as much was required to obtain the same degree of alveolar growth as when estrone was simultaneously administered. Estrone also markedly enhanced the ability of pituitary material to elicit alveolar growth. Estrone in large amounts inhibited the activity of progesterone on the mammary glands but did not inhibit the activity of pituitary materials. It was found that estradiol benzoate and diethylstilbestrol could be substituted for estrone as a synergist with progesterone to induce mammary lobule-alveolar growth. R.P.R.

569. Growth of the Male Guinea Pig Mammary Gland with Diethylstilbestrol. A. A. LEWIS AND C. W. TURNER, Univ. Mo. Endocrinol., *30*, No. 4: 585. April, 1942.

Groups of 3 to 5 male guinea pigs were injected subcutaneously daily with 0.01 to 50 micrograms of diethylstilbestrol. No obvious signs of main duct growth were apparent; however, some extension of the duct system appeared to have occurred at dose levels of 0.1 microgram and above. Lobule growth occurred in all groups showing duct extension. The early formed lobules contained no alveoli but when developed further alveoli were numerous. The development of male mammary glands did not compare favorably with that secured in a single treated female. Secretion was not observed in the male glands with alveoli. The teats of male guinea pigs in all groups were larger than those of the controls. Doses of diethylstilbestrol higher than 0.1 microgram caused no greater growth than did 0.1 microgram. R.P.R.

MISCELLANEOUS

570. The Treatment and Disposal of Waste Waters from Dairies and Milk Products Factories. Technical Paper No. 8. Water Pollution Research Board of Great Britain, His Majesty's Stationery

Office, London. (In U. S. A. can be obtained at British Library of Information, 30 Rockefeller Plaza, N. Y.)

The publication of this investigation on the disposal of dairy wastes will be welcomed in this country as much as in Great Britain where the work was conducted. Some years ago the Water Pollution Research Board was faced with the problem of devising a satisfactory method of purifying the polluting waste washing waters from dairy plants. An intensive investigation was initiated which covered a period of seven years. The experiments made, and the results obtained, with the conclusions and recommendations derived from them, are described in this report. These results should give confidence and direction to anyone confronted with the dairy waste problem, since a practical and economical method of waste treatment has now been designed for the special needs of our industry.

In reading the report one is impressed with the scope and thoroughness of the investigation. Two factors skillfully attended to in planning the investigation were no doubt responsible for the success attained in studying and appraising the various waste purifying processes. First, the investigation was conducted by a well-organized "corps of experts" including several engineers, chemists and biologists. Secondly, after preliminary laboratory and small-scale experiments, two experimental plants for work on a commercial scale were installed at a United Dairies milk and cheese plant. One treatment plant consisted of two percolating filters, each 25 feet in diameter, while the other plant was designed for treatment of milk effluents by the activated sludge process with aeration by bubbles of air passed through diffusers. The functioning of these plants was carefully studied for three years, and the results furnish much of the evidence presented in the report.

The results of the investigation showed that dairy wastes could be most satisfactorily purified by treatment in two percolating filters in series, with periodic change in the order of the two filters. By this method final effluents of high quality were obtained.

In general, satisfactory treatment of milk wastes by the activated sludge process was more difficult to control and the process was somewhat easily upset by flushes of liquid of abnormally high strength.

Plans, drawn to scale, of the double filtration plant and the activated sludge plant are included in the bulletin. There is a section devoted to a study of the organisms on the surface of the filters and those in the activated sludge. An appendix includes the methods of analysis of crude waste waters and treated effluents, notes on organisms isolated during the investigation and results of tests on the action of dairy waste waters on cement products.

J.H.E.

571. Paint in the Dairy Plant. W. K. HOFFMAN, Pittsburgh Plate Glass Co., Memphis, Tenn. South. Dairy Prod. Jour., *31,* No. 5: 24. May, 1942.

Directions are given for interior painting of dairy plant. White enamel consisting of resin and little or no oil are preferable to those containing substantial amounts of oil. The first type requires a foundation flat white coat for adhesion. One-coat products become yellow upon exposure as the oil which they contain oxidizes.

In cases where gray, black or orange spots of mold, fungus or "mildew" occur, the thorough washing of all surfaces is necessary. Trisodium phosphate or other equally effective compounds should be used. After this, the surfaces must be sterilized with products which liberate available chlorine to prevent the growth of fungus beneath or through the coats of paints which follow. A two-coat fume-resistant finish, free from oils capable of supporting mold growth and containing anti-mold compounds should follow. Strong poisons, such as bi-chloride of mercury should be avoided. It is desirable to apply to the dry painted surface a wash coat of clear fungicide as an added precaution. Application may be made with calcimine brushes or sponges. Such treatment should prevent growth of mold into the paint film, but growth in food deposits upon the surface may occur.

Maintaining paint on concrete floors is a very difficult problem. The presence of moisture beneath the floor and the dusting of concrete cause most failures. Old paint which is peeling should be entirely removed. The use of tri-sodium phosphate cleaning solutions and, if grease or oil is present, free alkali cleaners are recommended. Following thorough washing with clear water, the surface should be etched with a solution of one part of muriatic acid and three parts of water.

The painting of refrigerator pipe lines before peeling and scaling occur is recommended. Paints and primers of the zinc chromate bakelite type are vastly superior to iron oxide or red lead products.

Light-colored paints low in degree of gloss provide the maximum distribution of light and minimum of glare. F.W.B.

572. Trends in Dairying by Major Type-of-Farming Regions. W. F. FINNER AND R. L. MIGHELL, U. S. Dept. Agr., Technical Bull., *751.* Jan., 1941.

The principal purpose of the investigation here reported was to examine recent regional trends in dairying in the major type-of-farming regions of the United States. This was an initial step in a more complete analysis leading to careful estimates of probable long-time supply responses or trends for each region, under each of several possible sets of price relationships.

For many years there has been a marked and fairly continuous upward trend in milk production and in the number of milk cows in the U. S. This report examines the changes since 1928 by major type-of-farming regions. The Cotton Belt showed the highest rate of increase in number of milk cows from 1928 to 1939; this region, the corn belt, and the dairy region account for about 65 per cent of the total increase in milk production for the period. It is evident that much of the additional milk production in the Cotton Belt and in some of the other regions has been consumed locally, and that much of the increase in the Cotton Belt was on farms with small herds (3 cows or less).

Changes in the agriculture of selected areas have been examined for an explanation of the changes in dairying. In northern Vermont increases in production have been explained on the basis of increases in available feed as a result of improved fertilizer practices on grass and hayland. Dodge County, Wisconsin, also has increased dairy production largely through production of more feed on additional cropland and by growing more legumes. In the south it appears that dairy production is related to shifts out of cotton into feed crops and pasture, to reduction in the number of horses and mules, to the use of cropland not previously in production, and to the establishment of dairy manufacturing plants. J.G.A.

573. **Care of Equipment.** E. H. Forster, Cherry-Burrell Corp., Cedar Rapids, Iowa. Ice Cream Field, *39*, No. 5: 13. May, 1942.

The author considers the following of paramount importance for the proper maintenance of equipment. (1) Definitely assign responsibility for maintenance of each machine. Unless this is done proper lubrication and other necessary operations are not likely to be performed. (2) Inspect machinery at regular intervals. This should be done by one or more experienced and responsible persons. (3) Keep complete accessible file of installation, instruction and maintenance manuals and parts lists. (4) Keep complete file of data contained on name plates of all machines, motors, electrical starting equipment and miscellaneous equipment used. Attach this data to the parts lists. (5) Follow manufacturers, lubrication instructions to the letter. Considerable emphasis is placed on the importance of proper lubrication. (6) Make mechanical adjustments regularly. Do not wait until machine operation is affected. The cause should be determined before attempting major adjustments. (7) Maintain reasonable stock of replacement and repair parts. (8) Know your electrical equipment and get professional help on care and maintenance if necessary. (9) Train plant personnel in habits of careful handling of machine parts and provide equipment that will permit and encourage this practice. (10) Keep machinery properly cleaned.

The author states that machinery manufacturers, aware of the importance of proper maintenance, are helping plant operators and superintendents in the proper maintenance of their machinery. W.C.C.

574. Care of Equipment. A. W. FARRALL, Creamery Package Mfg. Co., Chicago, Ill. Ice Cream Field, *39*, No. 5: 13. May, 1942.

Accompanying the author's discussion are the following "ten commandments": (1) Read the manufacturer's instruction manual. The operator as well as the plan superintendent should do this. (2) Observe a regular lubrication program, using the correct lubricant for the job. (3) Train the operator so that he understands the principle of operation of his machine. Do this before he has done unnecessary damage. (4) Don't drop parts on the floor. Use a rubber or composition mat for supporting washed parts. (5) Don't overload the equipment. (6) Replace worn parts before they actually give out and cause breakage of other parts. (7) Provide good support for sanitary pipe lines. (8) Don't pound sanitary fittings. Use a wrench. (9) Follow the manufacturers' recommendations regarding cleaning procedure. Don't dump washing powder in the bottom of a vat. (10) Keep brine properly neutralized and treated so that it is not corrosive.

Emphasis is placed on proper sterilization by heat or chemicals and brief instructions given in each case. Special instruction is also given for the care of ice cream freezers, homogenizers and refrigeration equipment.

W.C.C.

575. Care of Equipment. GEORGE D. ARMERDING, Mojonnier Brothers Co., Chicago, Ill. Ice Cream Field, *39*, No. 5: 12. May, 1942.

The author gives the following "commandments," and briefly but effectively discusses and illustrates each: (1) Keep equipment clean. (2) Do not abuse your equipment. (3) Lubricate thoroughly and intelligently. (4) Practice regular inspection and prompt repairs. (5) Organize your efforts. (6) Conserve available material. (7) Provide proper tools. (8) Follow instruction manuals. (9) Study available substitutes. (10) Use some common sense. W.C.C.

576. Distribution: Trucks, Gasoline, and Labor. ARTHUR C. BUTLER, Automobile Mfrs. Assn., Washington, D. C. I. A. I. C. M. Prod. and Lab. Council Proc., p. 69. 1941.

Motor transportation problems in relation to national defense were discussed in this paper. Necessity for maintenance of transportation, heavy and medium trucks, replacement material, priority ratings, and rubber shortage and the problems they involve were explained and emphasized.

P.S.L.

577. Efficient Utilization and Substitutes for Cork. L. E. COVER, Armstrong Cork Co., Lancaster, Pa. I. A. I. C. M. Prod. and Lab. Council Proc., p. 55. 1941.

When cork board was placed on the mandatory priority system in June, 1941, defense requirements were given first consideration and food preservation at temperatures lower than 20° F. given next preferential rating. Allocations for repair and maintenance have been small and vary from month to month. Used with corkboard in truck bodies Temlok and Fiberglas have proved relatively efficient. The same materials may be used in ice cream cabinets and ¾ # density Fiberglas used in such nonstructural pieces as side walls, ends, and tops for cabinets. A method of testing these substitute insulators was described. P.S.L.

578. Chemical Effects in Refrigerating Systems. E. W. McGovern, E. I. duPont de Nemours & Co., Inc., R. & H. Chemicals Dept. Refrig. Engin., *43,* No. 5: 276. 1942.

Factors considered as underlying chemical reactions in refrigerating systems are moisture, air, heat, metals, lubricants, and the refrigerants. Various combinations of these factors may be found in one refrigerating system and not in another. With air and water present as contaminating substances, untoward solvent and chemical effects may follow giving rise to such conditions as corrosion, "sludge" formation, copper transfer, freeze-ups, and gasket failures. In removing moisture, a common mistake made in vacuum treatment is failure to apply a high enough vacuum and to hold it long enough. Drying agents recommended are activated alumina, "Drierite" or silica gel placed in the liquid line. Alkaline drying agents react with halogenated hydrocarbon refrigerants forming chlorides. Calcium oxide should not be employed in moist sulfur dioxide systems, as the rapid reaction between alkali and acid accompanied by evolution of large quantities of heat might give rise to an explosion. Perchlorate drying agents such as magnesium and barium perchlorates, should never be used in refrigerating systems, as serious explosions may result from their contact with lubricating oils or flammable refrigerants. Calcium chloride as a drying agent is undesirable since it may form a water solution which, in passing through the refrigerating system, will bring about corrosion.

To prevent sludging of oil used for lubrication in refrigerating systems, the oil must have high thermal stability and be not subjected to chemical and physical effects of excessive temperature, air drawn into the system, and moisture. Gaskets should be resistant to solvent action of oil and refrigerant as well, and for other than ammonia and sulfur dioxide systems in which rubber may be used, several of the newer products such as neoprene and the polyvinal alcohol plastic are finding use. L.M.D.

579. Fiberglas Insulation in the Low-Temperature Field. John B. Schneller, Equip. Insul. Div., Owens-Corning Fiberglas Corp. Refrig. Engin., *43,* No. 5: 280. 1942.

A description of the new "Fiberglas AE" board for low temperature and roof insulation is given. Research, stimulated by lack of cork imports, has led to the development of a desirable form of insulation board from the basic material, Fiberglas, one which lends itself to installation methods similar to those which have been in force for cork board. The basic "Fiberglas" offers high resistance to heat transfer, is inorganic, free of odor, does not absorb odor, is incombustible, will not rot, mildew, or offer sustenance to vermin. "AE" board is made of pure glass fibers, compressed to a density of 6 lb. to the cubic foot, and treated with a binder to increase the rigidity of the final product. The core of glass fibers is enclosed in a $\frac{1}{8}$-inch thick sheath of durable asphalt that has a high melting point. Surface treatment consists of white sand, rolled into the asphalt. This provides a good bonding surface and prevents adhesion of the boards to one another in transit or storage. The heat conductivity of the board is 0.265 BTU per sq. ft., per ° F., per inch of thickness at a mean temperature of 60° F. Its moisture absorptive property is in the lower range. It is fire retardant, and is resilient. L.M.D.

580. Opportunities of Reducing Operating Costs. (C) Distribution Cost. V. R. Corrigan, Polk Sanitary Milk Co., Indianapolis, Ind. Internatl. Assoc. Milk Dealers, Assoc. Bul., *34*, No. 15: 337–341. Feb., 1942.

Experience with the six-day delivery plan revealed that construction of more storage space, larger number of bottles needed and other extra expenses nullified any delivery savings during the early stages. Inadequate supply of Grade A milk for Friday processing also presented a problem. Although the six-day plan has merit and should ultimately effect savings, still all angles should be examined and prepared for before adopting it. The loyalty and confidence of the salesmen is the first step in improving distribution efficiency. The "Standard Order Method" reduces the temptation to embezzlement while the added clerical work of the system may be transferred from route salesmen to a clerical force with savings. From the records a "break even point" should be calculated for the route. E.F.G.

581. The Effect of Government Regulation of Retail Credit upon the Dairy Industry. M. M. Douglas, Credit Mgr., Maplehurst Farms, Inc., Indianapolis, Ind. Milk Dealer, *31*, No. 7: 34, 66–70. April, 1942.

The author points out the following facts: We must recognize the existence of an activity of certain forces which, under the guise of patriotism, are trying to reconstruct our national economy to fit their version of an economic Utopia. We must not lose sight of the fact that even though we are at war, there are still pressure groups of various sorts who have axes to grind. Under cover of the confused thinking and hysteria prevailing in times like these, they will try, by vigorous flag waving, to put over a few fast ones. And some of these individuals are in positions of great authority. The author discusses the effect of government regulation of retail credit upon the dairy industry. The results of the restrictions are summarized as follows: 1. Under every-other-day delivery the driver's opportuity to collect is cut in half. 2. Increased loads due to consolidation of routes, where that has been done, limits the amount of time available for collection. 3. The urgent necessity for conserving rubber prevents the possibility of extra trips to collect. 4. Previously contracted obligations, increasing cost of living, increasing taxes and the necessary purchase of defense bonds and stamps, mean that the customer, defense worker or otherwise, has a much harder time to make the budget balance.

Suggestions offered to overcome these various factors are as follows: First: We can keep our heads—this is no time to become panic-striken or hysterical. Common sense and straight thinking will accomplish many things. Second: We can establish community credit policies and cooperate. There is no competition in credit, so there is no reason why we cannot get together in our individual communities and work for the common good. Carried a step farther, we can work with our fellow dairymen in other cities. Third: We can tighten up our individual policies and keep our accounts as near current as possible. Fourth: Through our national, state and local trade associations, we can make a determined effort to secure representation at any conference or hearing held by the W.P.B. affecting us, directly or indirectly. Fifth: As one of the prime factors in the promotion of national health and economy, we can ask for serious consideration of our representations to the board. C.J.B.

582. **Preventive Maintenance on Trucks Pays Dividends.** ANONYMOUS. Milk Dealer, *31*, No. 7: 31–32, 60–61. April, 1942.

This article is a discussion of truck maintenance as practiced at the Chestnut Farms-Chevy Chase Dairy, Washington, D. C. A description is given of the daily inspection report on which drivers check items requiring repairs, adjustment, and attention. A copy of the general inspection form containing 53 items which the mechanic must check and okay every 2,500 to 3,000 miles, is shown. Other forms used, such as Trouble call, Wash report, Material requisition, Grease report, Daily electric report, Tire change record, etc., are also discussed. C.J.B.

583. **Cleaning and Sterilizing Dairy Plant Equipment.** C. M. MOORE, Diversey Corp., Chicago, Ill. Natl. Butter and Cheese Jour., *33*, No. 5: 10. May, 1942.

Use good cleaners properly: Rinse before washing; dismantle and scrub every piece of equipment and rinse with hot water after washing; special treatments may be necessary to remove milkstone. In washing cans, cleaners should: remove milk deposits, soften the water, keep the machine free of scale but should not remove tin.

Sterilization, the final step in cleaning, may be accomplished with steam or hot water at 180° F. for 2 to 5 minutes or with chlorine sterilizers which can be used effectively and economically even on open surfaces. Chlorine sterilizers containing 100 ppm. of available chlorine can be pumped through pipes, filters and heaters for about 5 minutes while large tanks and open surfaces are best sterilized by spraying with a 250 ppm. solution. Detailed instructions are given for each cleaning and sterilizing operation with special precautions for some equipment. W.V.P.

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ABSTRACTS OF LITERATURE

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ABSTRACTS OF LITERATURE

BOOK REVIEW

584. Chemistry and Physiology of the Vitamins. H. R. Rosenberg. May, 1942. Interscience Publishers, Inc., 215 Fourth Ave., New York. 674 pages, 25 figures; $12.00.

Recognizing the need for a comprehensive review of the chemistry and physiology of the vitamins, Dr. Rosenberg has presented in this book a concise, up-to-date and systematic review of all topics of vitamin research which have contributed to the rapid development and advancement in the knowledge of this field.

The opening chapter is a discussion of vitamins in general which includes definitions, historical development, nomenclature, list of vitamins and a general discussion of the topics which are followed systematically in every chapter on the individual vitamins.

In the chapters on the individual vitamins, there appears a concise review of nomenclature, historical development and occurrence. The discussions on the chemistry include methods of isolation, proof of chemical constitution and synthesis including space formulas for the various steps and industrial methods of preparation. There are special paragraphs on the biogenesis and specificity of vitamin action. The determinations of vitamins are subdivided into physical, chemical, biochemical and biological methods and are followed by a discussion of vitamin standards. The physiological treatment includes relationships in plant and animal physiology, the latter being subdivided into the metabolism of vitamins, the physiological action, mechanism of vitamin action and the relation of vitamins to each other, to hormones and minerals. The pathological aspect treats with avitaminosis, hypovitaminosis, hypervitaminosis and a special section on clinical tests. Finally the vitamin requirements are presented.

Following the chapters on the individual vitamins, there is a brief discussion of 17 non-identified vitamins. In the appendix is classified a group of essential compounds which are called vitagens. These include essential fatty acids, amino acids, carbohydrates, choline and related compounds and organic sulphur containing compounds.

In the patent index there are listed more than 1200 United States, British, German and French patents which are arranged chronologically according to subject matter. The essential data of each patent is given together with a brief abstract.

This book should appeal both to those engaged in vitamin research and to those who wish to be informed on this subject. It is well written and conveniently arranged for use as a reference text, with the bibliography readily available as footnotes. The reviews include literature through 1941 and even into 1942. S. M. Hauge

585. A Symposium on Respiratory Enzymes. University of Wisconsin Press, Madison, Wis. 1942. 281 pages; $3.00.

This book deals with the fundamental nature of those enzymes and enzyme systems that are closely connected with the action of vitamins. Each part of the book is written by an international authority in his field. Recent findings are discussed in detail and interpretations of most developments are made. The discussions on Phosphorylation, Respiration, and Hydrogen Transport which apply the fundamental findings to important, specific problems are especially informative and interesting.

The divisions on Intermediate Carbohydrate Metabolism by Meyerhof; Oxidative Mechanisms in Animal Tissue by Ball; Oxidases, Peroxidases and Catalase by Stern; Flavoproteins by Hogness; Cytochromes by Stotz; and Phosphorylation of Carbohydrates by Cori are especially well written. The sections on Pasteur Effect by Lipman; Nicotinamide Nucleotide Enzymes by Schlenk; Metabolic Cycles and Decarboxylation by E. A. Evans, Jr.; and Transamination by Cohen give information that is very timely.

All sections of the book carry good bibliographies and the book as a whole should serve as an excellent reference for individuals that are working directly in the field of respiratory enzymes. D. M. Doty

586. War Gases. MORRIS B. JACOBS. 1942. Interscience Publishers, Inc., 215 Fourth Ave., New York. 180 pages, illustrated; $3.00.

This book deals with the following general subjects in the field of war gases: 1. Chemical nature, physical characteristics and physiological response of the war gases. 2. Effect of war gases on materials, food and water and methods of obtaining samples of these for analysis. 3. Methods of detection of and analysis for toxic gases in foods, water, air and other materials. 4. Decontamination of various materials polluted by such gases.

The author stresses the importance of a complete knowledge of the problem, to intelligently and actively cope with any possible gas attacks in this country regardless of how remote they may appear at the present time. The information presented is of interest primarily to specialists in this field such as the war gas chemist, gas identification, decontamination and public health officers and air raid wardens.

Manufacturers and distributors of dairy products might well read those sections of the book dealing with effect of various gases on foods as well as discussions on removal of samples for analysis and methods of decontaminating after gas attacks. The author also states "The best way to obviate the necessity for salvaging foods after gas attacks is to protect them from contamination before the attacks." As is also emphasized, adequate protection must be based on a knowledge of the properties of the gases. Methods of protection must be known and put into practice before any possibility of attack exists in order to be sufficiently adequate to meet all emergencies.

This book is well written throughout. Many details regarding precautions and procedures are emphasized to the point of repetition. This is undoubtedly desirable from the standpoint of certain individuals not too familiar with all aspects of the subject but who yet are responsible for coping with any possible emergency. One fact very well brought out in the discussions is the complexity of the problems involved and the necessity for supervision of all gas defense efforts by experts in the field of gas warfare.

A publication of this size naturally could not include all of the useful information on the subject. The author therefore has preceded each chapter with general references. Frequent references are made in the text to other published investigations from which the author has drawn his information. The reader can thus obtain more detailed information on practically any phase of this subject. Complete author and subject indices also enhance the value of the book. P.R.E.

587. **Annual Review of Biochemistry, Volume XI.** Annual Reviews, Inc., Stanford Univ. P. O., California. 736 pages; $5.00.

The Annual Review of Biochemistry, as once before cited in the JOURNAL OF DAIRY SCIENCE book review, is an assembled group of reviews on selected biochemical subjects written upon invitation, by competent authorities. The Volume XI contains 28 sections and includes reference to some 3929 papers. A significant number of the reviews in this new issue are of real interest to those engaged in dairy products research. The Annual Review is written in technical research rather than popular terminology.

In X-Ray Studies of the Structure of Compounds of Biochemical Interest is presented a review of the background of knowledge concerning the details and principles of molecular and crystal architecture and experimental methods. Illustrations are employed to explain principles. Reference is made to studies on fibrous, corpuscular and virus proteins, carbohydrates and sterols, porphines and glycerides. The section on the Chemistry of the Acyclic Constituents of Natural Fats and Oils contains review of methods recently developed for the separation and identification of the individual and component fatty acids. Excellent discussion is presented on distribution and composition of acids, glycerides and phospholipids in vegetable, marine and animal fats. A very complete discussion is presented on the Chemistry of the Proteins and Amino Acids, and includes the size and shape of protein molecules, the number and configuration of the electrically charged groups in amino acids, peptides and proteins, and implication of the data for biochemical studies. The Metabolism of Proteins and Amino Acids is excellently reviewed. In two excellent chapters are considered the developments in the fields of the water- and fat-soluble vitamins. Since an immense flow of investigative work in the vitamin field is taking place, orientation and evaluation of the information, inferentially applicable to milk, is useful. Vitamins discussed in the first of the two sections include thiamine, nicotinic acid and nicotinamide, riboflavin, pyridoxin, pantothenic acid, choline, biotin, inositol, p-aminobenzoic acid, ascorbic acid, citrin; in the second, vitamins A, D, E, and K. The section on Calcium and Phosphorous Metabolism: Clinical Aspects, presents pertinent review on the relative availability of the calcium of milk, and on the mode of action of certain antirachitic factors. In the chapter on Biochemical and Nutritional Studies in Relation to the Teeth is included discussion on the significance of calcium and vitamin D in occurrence of dental caries. The nutritional demands of fowl is reviewed in Avian Biochemistry and is of value of interpreting better the nutritional adequacy in rations of milk by-products. In addition to the above chapters are: Biological Oxidations and Reductions; Hydrolytic Non-Proteolytic Enzymes; Chemistry of the Steroids, of the Hormones, of Visual Substances, of Muscle; Chemistry and Metabolism of Compounds of Phosphorous; Carbohydrate, and Fat, Metabolism; Lignin; Animal Pigments; Alkaloids; Mineral Nutrition of Plants; Plant Tissue Cultures, Immuno Chemistry, and Microbiology (including anti-bactericidal agents). The volume contains a subject and author index. Each section is provided with references cited in the review. K.G.W.

BACTERIOLOGY

588. Glutamine and Glutamic Acid as Growth Factors for Lactic Acid Bacteria.

MAXWELL A. POLLACK AND MANFRED LINDNER, Dept. Chem., Univ. Tex., Austin. Jour. Biol. Chem., *143*, No. 3: 655. 1942.

Nine lactic acid-producing bacteria are shown to require either glutamine or glutamic acid for growth. None of the bacteria tested is capable of producing glutamine from glucose and ammonium salts. V.C.S.

589. Routine Bacteriological Examination of Milk Cartons.

W. A. HOY, Natl. Inst. Res. in Dairying, Univ. Reading. Proc. Soc. Agr. Bacteriologists (Eng.), 48. 1941.

Of 1654 waxed paper pint milk cartons examined over a period of 6 years, 97% contained less than 200 recoverable bacteria per carton and 86% were virtually sterile. Of 20 cartons that gave counts over 600 (more than 1 per ml. of capacity), 16 were found to be crushed when they were unpacked. Milk souring organisms were found only occasionally. The results of the examinations show that waxed paper milk containers can be made and distributed to dairies in a satisfactory hygienic condition, and ready for immediate use. M.W.Y.

590. The Examination of Raw Milk by the Methylene Blue and Resazurin Tests and by the Plate Count. CONSTANCE HIGGINBOTTOM, Hannah Dairy Res. Inst. Proc. Soc. Agr. Bacteriologists (Eng.), 32. 1941.

Nearly 1,000 samples of raw milk from individual farms were examined by the plate count, methylene blue reduction test, and resazurin test. The samples were examined immediately on arrival at the laboratory and again after storage overnight at 40° F., and 60° F. On identically treated samples the correlation coefficient between the plate count and the methylene blue reduction test was high, *i.e.*, 0.84, 0.85, 0.90, and 0.73 (negative values) respectively. A very close agreement was obtained between the time taken to reduce methylene blue and to reach the vivid pink stage (Lovibond disc No. 1) with resazurin. M.W.Y.

591. The Micrococci of Milk. T. GIBSON AND Y. ABDEL-MALEK, Col. Agr., Edinburgh. Proc. Soc. Agr. Bacteriologists (Eng.), 31. 1941.

Pasteurization at 63° C. for 30 minutes of samples of certified milk and aseptically drawn milk destroyed all micrococci while those in milk of indifferent quality frequently survived the treatment. It is concluded that the cow's udder is not a source of the heat-resistant species. M.W.Y.

592. Resazurin Reduction of Fresh and Stored Udder Milk. S. B. THOMAS, Univ. Col. Wales, Aberystwyth. Proc. Soc. Agr. Bacteriologists (Eng.), 34. 1941.

Forty-four aseptically drawn samples of quarter milk from normal and latent mastitis infected udders were examined within one hour of milking, and portions of the same milk held at 5° C. and 15° C. were examined after 24 hours. Holding the milk for 24 hours resulted in considerable loss of resazurin-reducing activity. There was no significant difference in the reduction times of the portions held at the two temperatures. M.W.Y.

593. Cryophilic Bacteria as a Cause of Milk Samples Failing in the Methylene Blue Test. C. S. MORRIS, Scale-Hayne Agr. Col., Newton Abbot, Devon. Proc. Soc. Agr. Bacteriologists (Eng.), 33. 1941.

Samples of morning milk from a certain area failed to pass the methylene blue test after being held overnight in a refrigerator kept at 4° C. *Pseudomonas* types were isolated that grew rapidly in milk held at 4° C. Water supplies on some of the farms contained these *Pseudomonas* types and equipment became contaminated from these supplies. M.W.Y.

594. A Note on the Lipase of Some Lactic Acid Organisms. J. WOLF, Natl. Inst. Res. in Dairying, Univ. Reading. Proc. Soc. Agr. Bacteriologists (Eng.), 21. 1941.

Strains of *Lactobacillus casei, L. acidophilus, L. Lactis, L. delbruckii, L. helveticum, Streptococcus cremoris, S. thermophilus, S. mastitidis, S. mucosus, S. liquefaciens,* and *S. salivarius* were tested for production of fatty acids from butter fat to determine whether they are associated with production of flavor in cheese, where butyric acid and other fatty acids are known to occur. The organisms were grown in a peptone, Lemco, yeast extract, K_2HPO_4 medium (pH 6.9), to which 0.15% agar was added. 0.75% butter fat was added to half the medium. Incubation was at 38° C. and flasks were shaken daily for 7 months. None of the organisms produced fatty acids from the fat.

The possibility of an intracellular lipase was studied using *L. casei.* To digest the cell wall with proteolytic enzymes, a culture of *L. casei* was grown for 6 days in 1 liter of yeast dextrose broth. After centrifuging and washing the suspension aseptically, it was made up to 100 ml. and then divided into two 50-ml. portions. To one was added 2 ml. of a 5% pepsin solution and to the other 2 ml. of 5% trypsin, both of which had been passed through a Seitz filter. The trypsin suspension was brought to pH 8 and the pepsin one to pH 3 and incubated at 37° C. for 24 hours. 10 ml. of each of the digested-bacterial suspensions were placed in flasks and 30 ml. of the butter fat medium used previously were added as substrate. Controls containing the enzymes but no bacterial suspension were also included. The flasks were incubated at 28° C. for 2 months and were shaken daily. After examining each for sterility, 20 ml. from each flask were steam distilled in duplicate. The titrations of 100 ml. of distillate with N/100 NaOH from the tryptic bacterial suspension were 2.00 ml. and 4.80 ml. compared to 1.10 ml. and 1.05 ml. for trypsin only. A Duclaux distillation on the distillate from the tryptic bacterial suspension revealed that over 85% of the acid was butyric. *Lactobacillus casei* contains a lipase or butyrinase which is liberated on disruption of the cell. It is possible that the reason why butyric acid does not appear in cheddar cheese until it is mature is that the butyrinase is liberated at a late stage in ripening. M.W.Y.

595. **Resazurin Reduction as an Index of Milk Can Sterility.** S. B. Thomas, Univ. Col. Wales. Proc. Soc. Agr. Bacteriologists (Eng.), 47. 1941.

A series of 154 twelve gallon cans were picked at random after steaming at four creameries, held at atmospheric temperature for 24 hours and rinsed with 100 ml. of quarter strength Ringer's solution. Five ml. of rinsing solution was added to 5 ml. of sterile milk, mixed and incubated for 18 hours at 15° C. The resazurin test was then made. A comparison showed that 90% of the cans with colony counts above 100 per ml. of can capacity reduced resazurin within 1 hour whereas 97% of the rinsings taking over 5 hours to reduce resazurin to the pink stage had colony counts of 10 and under per ml. of can capacity. M.W.Y.

BREEDING

596. Dairy Cattle Breeds. A. B. Nystrom. U. S. D. A. Farmers' Bul. 1443. Feb., 1942.

This most recent revision of a familiar publication differs from its predecessors of 1935 and 1938 only in bringing up-to-date the number of cows of the different breeds in this country by sections; the milk and butter fat records of the ten highest producers in the United States of each of six breeds of dairy cattle; and in an increased amount of space given to the requirements for recording cows and bulls in the American Dairy Cattle Club.

J.G.A.

CHEESE

597. A Note on the Bacteriology and Chemistry of Portuguese Sheep's Milk Cheese. E. R. Hiscox, S. J. Rowland, J. Wolf, and M. M. Jacob. Natl. Inst. Res. in Dairying, Univ. Reading. Proc. Soc. Agr. Bacteriologists (Eng.), 24–26. 1941.

Examination of 8 cheese varying from 77 to 110 days of age showed that the ripening of Serra cheese like cheddar depends largely on the homofermentative lactobacilli. The volatile acids and the products of the nitrogen breakdown also resembled those of a cheddar of approximately the same age. M.W.Y.

598. Some Observations on Commercial Starters. J. Harrison and J. Z. Wolf, Natl. Inst. Res. in Dairying, Univ. Reading. Proc. Soc. Agr. Bacteriologists (Eng.), 19–20. 1941.

About 140 samples of starters were examined to determine the cause of fluctuations in acid production. Over 100 cultures behaved normally. Young, 18-hour milk cultures were tested in separated milk using 1% inoculum and incubating at 30° C. Two lots of milk were inoculated, one being the control and the other having two drops of whey added. Titrations of 10-ml. portions were made after 2- and 7-hour incubation. Over 60% of the normal starters produced less than 0.35% lactic acid in the 5-hour period under observation. Most of these "sluggish" starters originated from creameries where raw milk was used for cheese making. When the "sluggish" starters stood at room temperature overnight, after the 7-hour titration, and were titrated in the morning according to the usual practice, the acidity was normal. This titration is therefore not indicative of any abnormality which may occur in the starter.

When the addition of whey had an inhibiting effect upon acid production, only in six cases where the decrease was greater than 0.1% acid was it possible to demonstrate the presence of bacteriophage.

Although bacteriophage might be responsible for many cases of "slowness" where raw milk is used together with a "sluggish" starter, "slowness" may be due to a sudden change in the milk flora or the introduction of milk deficient in acid-producing organisms. M.W.Y.

599. The Problem of Bacteriophage in Cheese Starters. L. J. MEANWELL, United Dairies Res. Lab., London. Proc. Soc. Agr. Bacteriologists (Eng.), 16–18. 1941.

Failure of mixed-strain as well as single-strain starters was frequently due to bacteriophage infection. Bacteriophage was demonstrated by less acid-producing power when 0.1% "slow" whey was added to a 1% inoculation of that culture in sterilized milk. With normal healthy cultures, after six hours incubation at 30° C., there was little or no difference between the control tube and the tube containing 0.1 per cent "slow" whey, but with the susceptible culture an appreciable drop in acidity was found.

Until some system has been devised whereby bulk starters can be completely protected from phage infection, multiple-strain rather than single-strain starters should be used. Phage infection is frequently air-borne and it may be desirable to use a building entirely separated from the cheese-making process for the preparation of starter. M.W.Y.

600. The Evolution of Gases During Cheese Ripening. E. R. HISCOX, Natl. Inst. Res. in Dairying, Univ. Reading. Proc. Soc. Agr. Bacteriologists (Eng.), 23. 1941.

The quantity of CO_2 produced by cheddar and stilton cheese corresponded with the periods of rapid growth, first of the streptococci and later of the lactobacilli. With the growth of the mold in stilton a third increase of CO_2 was noted. The development of CO_2 appears to be largely a direct result of bacterial or mold action. M.W.Y.

CHEMISTRY

601. Color Tests for the Detection of Alpha-Dicarbonyl Compounds Formed in the Autoxidation of Fats. EDWARD A. PRILL, McArdle Memorial Lab., Univ. Wis., Madison. Oil and Soap, *19,* No. 6: 107. 1942.

The tests developed by the author provide definite evidence for the occurrence in autoxidized unsaturated fats of compounds containing either the alpha-diketo (C–CO–CO–C) or the alpha-ketoaldehyde (C–CO–CO–H) group. The presence of alpha-dicarbonyl compounds was found in autoxidized ethyl linoleate, corn oil, cottonseed oil, olive oil and lard. Peroxides in autoxidized fatty materials could be destroyed by treatment with ferrous chloride without destruction of alpha-dicarbonyl compounds. V.C.S.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

602. Protein-aldehyde Plastics. Combination of Formaldehyde with Acid Casein and with Rennet Casein. D. C. Carpenter and F. E. Lovelace, N. Y. Agr. Expt. Sta., Geneva. Jour. Ind. Eng. Chem., Ind. Ed., *34,* No. 6: 759. June, 1942.

Forms of casein-aldehyde plastic have been manufactured for many years, much of it reaching markets in this country in the form of buttons and buckles. In the manufacture of the plastic the casein is hardened by formaldehyde and it is this chemical reaction with which the authors are primarily concerned. Acid and rennet casein are different from each other in the binding of formaldehyde and their combining ratios are established over a concentration range up to 6.63% formaldehyde. The general law applicable to both types of casein, relating bound formaldehyde to total formaldehyde employed, is shown to be the adsorption law, $x = KC^n$, in the concentration range investigated. About 30% more aldehyde appears to be bound than can be accounted for on the basis of combining with α-amino, side chain amino and amide groups. Possible types of structure are discussed and models are given. B.H.W.

DISEASE

603. Cystic Pituitary in Young Cattle with Vitamin A Deficiency. L. L. Madsen, S. R. Hall, and H. T. Converse, Bureaus of Animal Inds. and Dairy Inds., U. S. D. A., Beltsville, Md. Jour. Nutr., *24,* No. 1: 15. July, 1942.

In a series of 15 animals of the beef and dairy breeds, varying in age from one day from birth to 787 days of age, which had either vitamin A deficiency at death or a previous history of vitamin A deficiency, 13 had cystic pituitary glands. The cysts occurred either in the residual lumen or within the posterior lobe, often causing compression of the gland and injury to the glandular parenchyma.

The high incidence of cystic pituitary glands in vitamin A deficient cattle suggests that this is a part of the pathology of vitamin A deficiency. C.F.H.

604. Report of the Committee on Communicable Diseases Affecting Man. Paul B. Brooks, Internatl. Assoc. Milk. Sanit. Jour. Milk Technol., *5,* No. 3: 141. May–June, 1942.

The latest report available is for 1939. During this year the U. S.

Public Health Service reported a total of 27 outbreaks were attributed to fluid milk and cream and 8 to milk products. The committee with one exception omitted "one outbreak of undulant fever; two in which neither the name of the disease nor the means by which the milk was supposed to have been contaminated were stated; one bearing a notation: 'Milk or cream suspected'; and a so-called 'outbreak' consisting of one case of gastroenteritis."

Of the 27 outbreaks attributed to fluid milk and cream 4 were typhoid fever; one paratyphoid; 9 scarlet fever and septic sore throat together; 2 bacillary dysentery; and 11 gastroenteritis (including food poisoning).

Of the 8 outbreaks attributed to milk products 6 were of gastroenteritis (including food poisoning), one typhoid and one paratyphoid fever.

Results are also given for the five-year period 1935–1939, inclusive. During this period the total number of outbreaks traced to fluid milk and cream was 175. The committee omitting several "outbreaks" of one case each as they did for 1939. Of the 175 outbreaks scarlet fever and septic sore throat accounted for 68; typhoid fever 62; gastroenteritis 36; bacillary dysentery 5; paratyphoid 3; and diphtheria one.

During this period 25 outbreaks were attributed to milk products. (Three being omitted, one an "outbreak" of only one case and two in which ice cream was reported as suspected, but without explanation.) Of the 25 outbreaks, 22 were of gastroenteritis, one of septic sore throat, one typhoid fever and one paratyphoid fever. Thirteen were charged to ice cream, eight to cheese, two to cottage or cream cheese and two to buttermilk.

A study made of the outbreaks due to fluid milk and cream reported in 1937, 1938, and 1939 with reference to the size of the communities in which the outbreak occurred showed there were a total of 95 outbreaks. Of these 82% occurred in communities of 25,000 or less. L.H.B.

605. The Eradication of Streptococcic Mastitis by Treatment with Tyrothricin. F. E. Martin, W. Chester, Pa. Amer. Vet. Med. Assn. Jour., *101,* No. 784: 231. July, 1942.

With an average of 2.3 treatments per quarter, 117 of 130 infected quarters found in 49 of 71 cows were freed of infection. Twelve of the 49 were not completely freed of the infection, while seven cows "with more or less permanent defects of the natural barriers to infection" became reinfected during the winter. No reinfection occurred when cows were on continuous pasture.

Diagnosis was made from smears of incubated samples. Segregation was not practiced nor were precautions taken against spreading infection to healthy cows. Treatment consisted of injecting 20 cc. of a 50% emulsion of mineral oil containing 1.5 mg. of tyrothricin per cc. into the quarter immediately after milking and removing it at the subsequent milking. Infected quarters were examined immediately after treatment and treatment repeated until at least two negative tests at bi-weekly intervals were obtained. Cows were treated in all stages of lactation. S.A.F.

606. **Field and Laboratory Observations on Mastitis.** P. M. F. Shattock, Natl. Inst. Res. in Dairying, Univ. Reading. Proc. Soc. Agr. Bacteriologists (Eng.), 43–44. 1941.

An attempt was made to eradicate *Streptococcus agalactiae* from two herds of 89 cows. Difficulty in controlling the disease at first was partly due to the high incidence of 21% infection in animals in their first lactation. A further factor was the choice of bacteriological technique, since infections detectable only by enrichment methods may be present for many months before detected on Edward's medium. After the herds became virtually free from *S. agalactiae* infection, high proportions of both hemolytic and non-hemolytic staphylococci were found in many cases. The average percentage of cows excreting non-hemolytic staphylococci in Herd A was 19% and in Herd B 38% while non-hemolytic staphylococci were excreted by 9% and 40% respectively. Subclinical infection with hemolytic staphylococci may give rise to very appreciable alteration in the chemical composition of the milk. Staphylococci may be an unsuspected factor in causing milk to fall below the legal chemical standards. M.W.Y.

FEEDS AND FEEDING

607. **Hay Crop Silage.** N. N. Allen and J. B. Fitch. Minn. Agr. Expt. Sta. Bul. 360. May, 1942.

A popular bulletin, giving an account of the work done with grass silage at the Minnesota station, setting forth the advantages and disadvantages of this system of storing crops, and containing numerous recommendations and suggestions for the making of grass silage. J.G.A.

608. **Feeding Grass Silage.** C. B. Bender. N. J. Agr. Expt. Sta. Bul. 695. May, 1942.

A popular bulletin outlining the advantages of cropping systems which include grass silage and giving results of some of the more recent feeding trials with this type of silage at the New Jersey station. Points stressed are the need for accustoming farmers and cows to this comparatively new type of roughage, the economy of feeding it, and its favorable effect on milk flavor. J.G.A.

FOOD VALUE OF DAIRY PRODUCTS

609. **Composition and Thiamin and Riboflavin Content of Defatted Milk**

Solids. C. M. O'Malley and E. J. Baldi, Amer. Dry Milk Inst., Inc., Chicago, Ill. Jour. Milk Technol., *5*, No. 3: 138. May–June, 1942.

Thirty-two samples of defatted milk solids from 13 states representing the major milk-producing areas were analyzed during the spring of 1941.

The riboflavin and thiamin content of the samples tested were quite uniform, and there was no apparent relationship between the composition and vitamin content; nor did the geographic origin of the samples show any relationship.

The mean thiamin content of all samples was 3.57 micrograms per gram. The mean riboflavin content was 20.93 micrograms per gram. L.H.B.

610. Nutritional Requirements of Children. A Résumé. William J. Dann and Wilburt C. Davison, Nutr. and Ped., Duke Univ., School of Med., Durham, N. C. Amer. Jour. Dis. Children, *63*, No. 2: 366–370. Feb., 1942.

The authors, in this review, show in table form the daily allowances of the various dietary essentials for children. They state that these allowances are ample for healthy children of average size and activity with normal alimentary absorption but must be increased if the child is exceptionally large or active or has fever. Also care should be taken that the vitamins are not destroyed by excessive cooking.

The authors point out that milk, eggs and meat are excellent protein sources of the ten amino acids which are indispensable in the diet. B.E.H.

611. Prevention of Dental Caries by Massive Doses of Vitamin D Ralph Howard Brodsky, Bela Schick, and Hermann Vollmer, Sea View Hospital, New York. Amer. Jour. Dis. Children, *62*, No. 6: 1183–1187. 1941.

A group of 101 children who had tuberculosis or had been in contact with a patient with the disease were studied carefully for a period of one year to determine the effect of a single massive dose of vitamin D on the incidence of caries. They found that as the dose of vitamin D increased, the number of cavities in the teeth decreased. They divided the 101 children into 3 groups: A, control, hospital diet only; B, 305,000 U.S.P. units of vitamin D and 2,455,000 U.S.P. units of vitamin A in 30 cc. of fish liver oil concentrate in addition to hospital diet; and C, 600,000 U.S.P. units of vitamin D in form of crystalline vitamin D_2 in 1 cc. of oil in addition to the hospital diet.

They conclude, "Further studies are required on larger groups. However, the results of this study seem to indicate that the incidence of dental caries may be markedly decreased by the administration of a single massive dose of vitamin D." B.E.H.

612. Level of Vitamin A in the Blood as an Index of Vitamin A Deficiency in Infants and in Children. J. M. Lewis, O. Bodansky, and C. Haig, Dept. Pediatrics, Beth Israel Hospital, New York. Amer. Jour. Dis. Children, *62*, No. 6: 1129–1148. 1941.

The authors conclude that, "Low levels of vitamin A in the blood in rats were found to be associated with low intakes of the vitamin and with little or no storage in the liver." They also state that 45 U.S.P. units of vitamin A per 100 cc. was the lowest found in the blood of 144 normal infants. They found that the administration of 17,000 units of vitamin A to infants during the first 6 months of life increased the vitamin A content of the blood. However, no effect on the level of vitamin A in the blood was noted when infants of 6 to 18 months old were given the vitamin A preparation for periods of 1 to 5 months. The authors are of the opinion that the level of vitamin A in the blood may be a more sensitive indicator of vitamin A deficiency than the result of the dark adaptation test. B.E.H.

HERD MANAGEMENT

613. Report of the Committee on Dairy Farm Methods. Horatio N. Parker, Internatl. Assn. Milk Sanit. Jour. Milk Technol., *5*, No. 3: 152. May–June, 1942.

A discussion of the fundamentals of clean milk production and dairy farm inspection is reported.

An important thing pointed out was "the unwisdom of different health departments prohibiting practices that are not objected to by others, such for instance as stipulating that covered pails should or should not be used, that milk houses should be located at certain distances from dairy barn, that surface coolers may or may not be used, etc." To avoid confusing the farmers, inspection methods should be unified. L.H.B.

614. The Progress, Results and Cost of Electric Refrigeration on Dairy Farms. L. M. Graves, M.D., and Rex D. Bushong, D.V.M., Superintendent, and Director, Bureau of Sanitary Engineering, Dept. Health, Memphis, Tenn. Jour. Milk Technol., *5*, No. 3: 131. May–June, 1942.

Mechanical refrigeration has resulted in improved quality of milk supplies. Electrically operated units were found to be more economical than gasoline-powered units. The estimated cost of electrical refrigeration varied from 6.6 cents to 12.72 cents per hundred pounds of milk.

Eighteen cities out of 28 replying to a questionnaire, reported that 50% or more of the dairies in their respective milk sheds were using electrical refrigeration for cooling. L.H.B.

615. The Use of Sodium Hypochlorite for Routine Disinfection in the Cow Shed. J. HARRISON AND A. T. R. MATTICK, Natl. Inst. Res. in Dairying, Univ. Reading. Proc. Soc. Agr. Bacteriologists (Eng.), 41–42. 1941.

When hypochlorite is added to wash water, *Streptococcus agalactiae* is killed with a reasonable margin of safety so long as the concentration of available chlorine remains above 250 p.p.m. with an exposure time of 10 seconds or over. The exposure time for the udder cloth is assured by using two cloths alternately. Determination of the rate of decline of concentration of available chlorine in wash water used for washing down the animals and in water used for dipping milking machine teat cup clusters between milkings of individual cows led to the following suggested routine.

Use two gallons of a hypochlorite solution of 800 p.p.m. available chlorine. This is enough for about 12 cows. Two buckets must be used, one containing 2 cloths for washing down the animals, the cloths being used alternately. (The one not in use is left in the disinfectant water.) The other bucket should contain 2 cloths for wiping.

After milking a cow and before starting to milk another rinse the teat cup cluster in a bucket of clean cold water, drain for 5 seconds, dip into a 2 gallon bucket of water containing hypochlorite (800 p.p.m. is sufficient for 24 teat cup clusters), plunging 3 times in 4 seconds. Drain for 3 seconds. M.W.Y.

616. The Effect of Steam, Hot Water, and Chlorine on the Life of Milking Machine Rubbers. W. A. HOY AND F. K. NEAVE, Natl. Inst. Res. in Dairying, Univ. Reading. Proc. Soc. Agr. Bacteriologists (Eng.), 39–40. 1941.

The life of liners steamed for 5 minutes was about 8 weeks, and similar results were obtained by steaming for 2 minutes. In every instance the bacteriological tests of the steamed units and the milk were satisfactory. In the case of hot water treatments, 160° F. for 10 minutes was the least destructive to the rubber but bacteriological results were not satisfactory. A temperature of 170° F. for 10 minutes gave about the same length of life as 2 minutes and 5 minutes steaming, and the results of the bacteriological tests were satisfactory. The life of liners, treated with a warm chlorine-soda solution as a combined cleaning and sterilizing process was 8½ weeks. Liners that were washed but not sterilized lasted 10 weeks. The bacteriological tests of the units treated with chlorine were occasionally unsatisfactory, but the tests of the washed units were always unsatisfactory.

It is concluded that effective sterilization by steam or hot water does not seriously reduce the life of rubber teat cup liners, and that steam sterilization is no more destructive than an efficient hot water treatment. Five minutes jet steaming is effective and allows a sufficient margin of safety in practice. M.W.Y.

617. **Dairy Goat Management.** G. VAN DER NOOT. N. J. Agr. Expt. Sta. Cir. 418. May, 1942.

A popular bulletin describing the four breeds of dairy goats of importance in the United States and giving specific directions for feeding and management. J.G.A.

ICE CREAM

618. **The Effect of Cultures and the Relation of Acid Standardization to Several of the Physical and Chemical Properties of Ice Cream.** W. H. E. REID AND L. E. SMITH. Mo. Agr. Expt. Sta. Res. Bul. 339. Jan., 1942.

The use of cultures to increase the acidity of mixes after partial neutralization was studied. Mixes were reduced in acidity from as much as 0.24 to as low as 0.06% in certain instances. Many photomicrographs of ice crystals are shown as well as many photographs of melting studies.

It was found that "cultured" ice cream had a desirable flavor and good keeping qualities. It is recommended that a starter mix be prepared by incubating at 74° F. until the acidity reached 0.85% acid, after which sufficient quantities could be added to the regular mix before pasteurization.

Standardizing the mix acidity below that which is normal for the mix requires more standardizing agent than is theoretically required, due to the buffering capacity of the mix ingredients. The standardization and subsequent development of the acidity in the mix did not affect the stability, nor the crystalline structure of the ice cream appreciably. The standardizing agent (Minsol) appeared to minimize shrinkage during storage of mixes containing 13 and 15% serum solids. C.D.D.

619. **Effect of Dextrose and Sucrose Sugars upon the Properties of Ice Cream.** W H. E. REID AND K. R. MINERT. Mo. Agr. Expt. Sta. Res. Bul. 339. Jan., 1942.

This bulletin describes the effect of the use of dextrose and sucrose on certain properties of ice cream mix. It is accompanied by many photomicrographs showing the structure of the resulting ice cream, ice crystals. as well as many photographs of ice cream subjected to melting tests.

The replacement of sucrose with variable increments of dextrose had no significant effect on pH, acidity, viscosity or the specific gravity of the ice cream mix. The samples containing the dextrose were slightly less sweet than the control samples, but was not particularly noticeable until a substitution of one-third of the sucrose was made.

Dextrose affected the resistance of melting and lowered the freezing point of the mixes.

When ice cream containing sucrose only was drawn from the continuous freezer at 22° F., the ice crystals were smaller than comparable samples containing dextrose. With each increment of dextrose it was found desirable to lower the drawing temperature of the ice cream. Dextrose did not affect the number of dishes of ice cream obtained per gallon. C.D.D.

620. Report of Committee on Ice Cream Sanitation. F. W. Fabian, Internatl. Assn. Milk Sanit. Jour. Milk Technol., *5*, No. 3: 166. May–June, 1942.

Andrew J. Krog, of Plainfield, N. J , a committee member, suggests the need of regulations governing the production of certain ingredients now being used in ice cream mix, namely, butter from plastic cream, low lactose milk solids, high conversion corn syrups and corn syrup solids, stabilizers, egg products and fruits and nuts.

A temperature of 40° F. should be considered as the maximum for mix storage.

Where novelty products are packaged in envelopes a mechanical arrangement should be required for opening the envelopes.

Wiping cloths may be sterilized in a 1–5000 solution of "Roccol" (alkyl-dimethyl-benzyl-ammonium-chloride). This product will not impart off flavors or odors to the product.

More adequate control of retail outlets should be practiced.

W. C. Cameron, Ottawa, Canada, reported on the ice cream situation in Canada—1941, as follows: Sales of ice cream showed a substantial increase over 1940; final statistics will probably show 1941 to be the largest on record from the standpoint of gallonage manufactured and sold. Preliminary test used for fat in ice cream is to weigh 9-gm. sample of melted ice cream into 20% ice cream test bottle, add 13 ml. of glacial acetic acid and 9 ml. of commercial sulphuric acid. If sodium alginate has been used as a stabilizer in the manufacture of the ice cream; increasing the amount of glacial acetic acid to 17 ml. will give a clearer reading. "Counter freezers do not appear to be gaining in popularity."

Ralph E. Irwin, Harrisburg, Pa., reported on developments in Pennsylvania concerning frozen desserts sanitation.

"Farms and plants contributing to preparation of ice cream are to meet the same sanitary standards as farms and plants for the preparation of milk."

A freezer may not be located in the sales room, restaurant, etc.

A small ice cream plant that may be built in sections has been prepared for use in the manufacture and sale of ice cream at county fairs. These plants consist of a wash room, freezing room and sales room. Facilities for heating water to be used in cleansing and sterilizing equipment are provided.

Trucks built in three parts with facilities listed above, are also available for use at county fairs. L.H.B.

621. Suggestions for Cleaning Equipment in the Plant. C. W. COUGHLAN, Natl. Electric Mfrs. Co., New York. Ice Cream Rev., *25*, No. 6: 74. Jan., 1942.

The least objectionable way of removing milk stone, which is principally casein and milk salts rendered insoluble by heat, is to use a weak organic acid, such as tartaric acid, with mechanical scrubbing friction. For cleaning coil vats use one-half pound of tartaric acid crystals to 12 gallons of water. This makes a one-half per cent tartaric acid solution.

Corn sugar is also a solvent of milk stone and can be used for its removal. J.H.E.

622. Vanilla—Preparation and Types. E. G. WEED, Foote and Jenks, Jackson, Mich. Ice Cream Rev., *25*, No. 7: 26. Feb., 1942.

The author describes the culture, harvesting and curing of vanilla beans, with special emphasis on Mexican vanilla, having first-hand information on this variety as a result of trips to the Vera Cruz region.

In Mexico the picking of the vanilla pods begins in November and carries through until February. At picking time the pods contain no vanilla flavor and no vanillin. The first picking of beans comprises the wind-falls, the immature, crooked and damaged pods and often equals 15 to 25% of the entire crop. These are known as cuts or Picadura grade and lack fine flavoring properties. Next comes the ordinary, composed of short, immature pods, woody in texture, and a light reddish-brown shriveled bean. Later comes the Medium Crop of fairly good flavoring quality, followed by the Buena, or good grade. These are thoroughly seasoned pods, long, plump and dark brown in color. The last crop comprise the Prime beans. These are first quality, being dark chocolate brown, almost black in color. They are long, broad and supple and carry a rich mellow bouquet.

The Indians bring the beans to the curing stations where they are sorted as to size and quality, after which they are packed in large containers, or sweat boxes, holding from 2000 to 4000 pounds. They are allowed to sweat and ferment, with alternate sweating and drying. This is the crucial stage in the preparation of the vanilla bean. Among other little-known changes, enzymic action changes coniferyl alcohol to vanillin. About five pounds of green beans are required to produce one pound of finished cured beans.

A cured vanilla bean contains fiber, water, resin, soluble and insoluble gums, fat, fixed oils, volatile oils, vanillin and other compounds. In extracting the flavor locked up in the beans the avoidance of heat is to be desired. The beans are chopped and packed in percolators where an alcoholic menstruum is pumped over the beans until the flavor is completely exhausted. J.H.E.

623. Sugars in Ice Cream. C. D. DAHLE, Pa. State Col. Ice Cream Rev., *25*, No. 9: 24. Apr., 1942.

Various corn sweeteners and honey can successfully be used to replace a part of the sucrose in ice cream mixes. The corn sweeteners are of different compositions and the effect on the freezing point and relative sweetness varies.

Regular corn syrup in ice cream has a sweetness of 30–40% of sucrose and the freezing point of a 15% solution is 30.55° F. Corn syrup solids are now available and the sweetness is about 50% that of sucrose. Enzyme converted corn syrup has a sweetness approximately 66% of sucrose. A 15% solution has a freezing point of 29.98° F. Corn sugar is 80% as sweet as sucrose and a 15% solution has a freezing point of 28.63° F. A number of formulas are given in which the various corn sweeteners, invert syrup and honey replace a part of the sucrose. J.H.E.

624. More Gallons Per Cabinet Hole. C. W. ESMOND, Gundlach Co., Cincinnati, O. Ice Cream Rev., *25*, No. 8: 29. Mar., 1942.

A successful sales promotion campaign of one ice cream company is described. Education must always start with the company employees. Education of dispensers is stressed also. Dealers and clerks are treated liberally with any new "feature flavor." The company also supplies hostesses to dealers to pass out samples of the feature flavor ice cream. When rainy days and slack season slow up sales, the company redoubles its efforts to help dealers by circularizing of neighborhoods with handbills. J.H.E.

625. Check upon Waste in Refrigeration. C. T. BAKER, Atlanta, Ga. Ice Cream Rev., *25*, No. 8: 28. Mar., 1942.

The danger and inefficiency of pumping ammonia liquid in a compressor is considerable. Compressor output is reduced and there is danger of damage to valves and pistons, particularly in high speed machines. Excessive piston ring wear results because of difficulty of maintaining adequate oil film on piston due to washing effect of the liquid. The appearance of liquid in the compressor discharge results in a decided drop in temperature of the discharge gas, which in turn causes a sudden contraction of flanged joints with a leaky joint likely resulting.

There are many causes for pumping liquid. The best way to detect it is to place a reliable thermometer at the suction intake of the compressor. If such a thermometer fails to indicate a few degrees of suction superheat, but indicates saturation temperature, one may be certain that liquid is present. J.H.E.

626. Use of Sweetening Agents in the Manufacture of Ice Cream. K. M. Renner, Tex. Tech. Col., Lubbock, Tex. Ice Cream Rev., *25*, No. 9: 44. April, 1942.

When the experimental evidence of the past few years on the use of corn sweeteners in ice cream is appraised, it is found that from 25 to 30% of the sucrose in the mix can be replaced to a definite advantage. While there is some variation between investigators regarding actual sweetening values of the various corn sweeteners, the actual variation is well within the limits of normal range.

The author is of the opinion that when the percentage replacement is from 25 to 30% of the sucrose, the pounds of other sweetener required to secure the same sweetening as one pound of sucrose is as follows: Dextrose 1.1, enzyme converted corn syrup (sweetose) 1.5, corn syrup solids 2, invert sugar solution 1, honey 1.28. J.H.E.

627. Sweetening Agents Suitable for Ice Cream. J. H. Erb, Ohio State Univ., Columbus. Ice Cream Rev., *25*, No. 9. Apr., 1942.

In replacing sucrose with various other sweeteners, the first problem encountered is that of comparative sweetness. The published results indicate no "blanket" or general value can be assigned to any sweetener. The relative sweetness appears to depend upon the concentration being compared and also the supplementary effect noted when two or more sugars are present in the same solution.

Formulas are given for replacing one-third of the sucrose with only sufficient corn sweetener to arrive at palatability and sufficient sweetness. In the case of standard corn syrup, it was found that 1.5 pounds would satisfactorily replace 1.0 pound of sucrose, when one-third of a 15% sugar mix was replaced with corn syrup. J.H.E.

628. Refrigeration in Ice Cream Factories and Other Dairy Plants. L. C. Thomsen, Univ. Wis., Madison. Ice Cream Rev., *25*, No. 6: 18. Jan., 1942.

Dairy plants have a choice of five major systems of refrigeration: (1) the direct expansion system, (2) the flooded type, (3) brine system, (4) the "sweet water" system and (5) the "sweet water" ice storage system. Each has certain advantages and disadvantages, which are discussed. The sweet water system has had much popularity in recent milk plant installations.

Calculations and charts are given proving that for greatest capacity and lowest operating costs the evaporating side of the system should be run with the maximum suction pressure possible, still obtaining the desired temperatures. A room may, under certain conditions, actually be cooled to a lower temperature more rapidly by increasing the temperature of the refrigerant in the evaporating coils, than by calling for more refrigeration by turning down the thermostat or by operating at a lower suction pressure. In addition, the cooling operation will cost less. This is because at higher suction pressures the compressor will handle a greater weight of gas with each displacement of the cylinder.

It is recommended that greater use be made of gauges and instruments in refrigeration to check on proper operation. J.H.E.

MILK

629. New Tests on Refrigerated Milk. JOHN E. NICHOLAS AND T. G. ANDERSON, Pa. State Col., State Col. Pa. Refrig. Engin., 44, No. 1: 23. July, 1942.

Bacteriological analyses in different strata in 10-gallon milk cans is a function of temperature. A 10-gallon milk can was fitted with sampling tubes located at three levels, top of breast, at middle of body, and near the bottom of the body. At each level three tubes were inserted to three depths, one at the geometric vertical center, one near the wall of the can, and one reaching to vertically midway between. This arrangement rendered it possible to remove samples of milk for temperature and bacteria determinations at the several levels. The bacteriological analyses reported covered three different sets of conditions under which the milk was cooled; namely, (a) with a large amount of initially available refrigeration and the water both in motion, (b) with a large amount of initially available refrigeration, but no motion of the water bath, (c) with both the milk can and water bath in motion simultaneously.

The most satisfactory results were obtained when a water to milk ratio of 6 to 1 was employed with a heavy ice bank and the water bath agitated, the temperature in the top layer of milk having been lowered from 90° to 46° F., while that of the bottom layer had reached 37° F. in one hour. During this time, the water bath had remained below 35° F. and continued to do so throughout the succeeding cooling hours. The average bacterial content in most instances decreased, the better results being obtained under the first condition of cooling.

The motion of can in addition to water-bath agitation would not seem to give any material advantage though a lower ratio of water to milk was employed and not as much ice bank.

Milk cooled under the condition of heavy ice bank, without bath agitation, but with a water bath to milk ratio of 6 to 1, while only reaching 50° F. in the top layer in 4½ hours, revealed very little change in average bacteria content of the milk after 15 hours in the cooler as compared to that of the fresh milk.

The results indicate that it is particularly important to reduce the temperature of the top layer of the milk to below 50° F. as rapidly as possible because it was found that there was a variation in the ratio between the organisms in the fresh milk and the cream layer from as low as 5.5 to 1 to as high as 173 to 1.

When homogenized milk was studied, it was found that the bacterial content remained practically the same under conditions in these experiments.

L.M.D.

630. Thermoduric Bacteria in Milk and a Simple Remedy Against Them.

M. J. Prucha, Dept. Dairy Husb., Univ. Ill. Milk Plant Monthly, *31,* No. 4: 38. April, 1942.

A large number of different species of bacteria belong to the thermoduric group, a group able to withstand in large part ordinary pasteurization exposures. While the general belief is that the problem of thermoduric bacteria is primarily on the farm, not all milk plants are innocent. Milk stone is invariably a source of contamination. The remedy to eliminate or reduce the number of thermoduric bacteria consists in (1) having good, smooth-surface equipment, (2) washing utensils and equipment properly so no bacterial food remains, (3) giving the utensils satisfactory bactericidal treatment, and (4) avoiding prolonged heating of milk at temperatures between 100° and 143° F.

G.M.T.

631. The Problem of Controlling Rancidity in Milk.

N. P. Tarassuk, Dairy Inds. Div., Univ. Calif., Davis, Calif. Milk Plant Monthly *31,* No. 4: 24. April, 1942.

Rancidity in milk may result from spontaneous or induced activity of lipase present in milk. Apparently two lipases exist in milk, the one present in late lactation milk which acts spontaneously and the other present in all milk which is activated by (1) prolonged and violent agitation of warm milk, (2) rewarming precooled milk to 86° F. and recooling to 50° F., and (3) homogenization. By warming the milk to 105° F. development of rancidity is prevented although the enzyme is not destroyed. The feeding of green feed to cows in late lactation remedies the difficulty with spontaneous development of rancidity. By mixing normal milk with "lipase milk" in the proportion of 2 to 1 before any rancidity develops prevents the development of rancidity.

G.M.T.

632. Preparation and Merits of Churned Buttermilk.

C. L. Roadhouse, Dairy Dept., Univ. Calif., Davis, Calif. Milk Plant Monthly, *31,* No. 7: 32. July, 1942.

A method for making a pleasing-flavored, churned, cultured buttermilk, containing butter granules, between 1 and 2 per cent of milk fat, and which does not "whey off" readily has been developed. The method consists basically in (1) pasteurizing the skim milk between 185° and 190° F. for 1 hour, (2) pasteurizing the cream separately at 145° F. for 30 minutes, (3) adding 0.75% fat in the form of cream along with approximately 1% starter, setting the milk at 70° F. and ripening to an acidity of 0.70 to 0.80% before churning, (4) churning at 68° to 72° F. by circulating the culture through a centrifugal pump back to the vat, (5) salting at the rate of 2.2 ounces salt to each 100 pounds of milk, (6) adding 0.025% sweet cream to the granuled product, and finally, (7) cooling the buttermilk to 40° F. or below. Butter color is added to give the granules a high color. Butter granules remain more evenly distributed if the buttermilk is not bottled until after it is stored several hours. G.M.T.

633. **Homogenized Milk.** G. M. TROUT, Mich. Agr. Expt. Sta., East Lansing, Mich. Milk Dealer, *31,* No. 10: 30–31, 51–53. July, 1942.

The problems incident to the production and use of homogenized milk are discussed under behavior changes and processing. The problems under behavior changes are classified as follows: 1. Processing. 2. Packaging. 3. Distribution. 4. Laboratory Control. 5. Cooking. 6. Utilization of Returns. The processing problems are: 1. Rancidity. 2. Sedimentation. 3. Increased Bacterial Counts. 4. Inefficient Homogenization-meeting Standards. 5. Cream Line. The following conclusion is made: While homogenized milk apparently gives rise to numerous problems, these problems fortunately may be solved. Let it be recognized that problems exist in the processing and distribution of any product and homogenized milk is no exception. The general acceptance of homogenized milk indicates that the problems are not insurmountable. C.J.B.

634. **The Use of Laboratory Pasteurization in Solving Milk Problems.** ELIAS B. BOYCE, HERMAN C. LYTHGOE, ELLA K. RUGGLES AND ROBERT LANE, Div. Food and Drugs, Dept. Health, Boston, Mass. Jour. Milk Technol., *5,* No. 3: 146. May–June, 1942.

Laboratory pasteurization with standard agar plate counts on the milk before and after pasteurization when compared with the plate count of the plant pasteurized sample will give a good indication as to the efficiency of the pasteurization process carried on in the plant.

In many cases of high bacterial count on pasteurized milk the cause has been found to be due to the presence of thermoduric organisms in the raw supply. Milking machines have been found frequently to be the cause of these organisms in the raw milk. Laboratory pasteurization was also shown to give a "clean bill of health" in some cases to the producer. Showing conclusively that the trouble was in the plant. L.H.B.

635. Chemical Taste in Milk. Chas. Paley, Certified Labs., Inc., New York, N. Y. Jour. Milk Technol., *5*, No. 3: 165. May–June, 1942.

Reporting an incident where the use of a chlorine rinse for dairy equipment caused a chemical flavor to be imparted to the first milk over the equipment. L.H.B.

636. Relation of Ascorbic Acid and of Oxygen to Oxidized Flavor in Milk. J. C. Leeder and E. O. Herreid, Vt. Agr. Expt. Sta., Burlington. Vt. Agr. Expt. Sta. Bul. 481. 1942.

The milk used in this study was from the university herd and averaged 3.85 and 3.91% fat during the two trials. Pasteurization was carried out in a 30- and a 1-gallon stainless steel vat. Cooling was done either in the pasteurizer or over tinned copper surface cooler.

Oxygen and ascorbic acid determinations and flavor observations were made on the pasteurized milk held for definite storage periods. The surface-cooled milks lost more ascorbic acid and developed more oxidized flavor than did those cooled in the pasteurizing vat.

The ascorbic acid content of the mixed herd milk was not altered by changes from winter to summer feeding but the oxidized flavor occurred more often during the barn feeding. The off flavor appeared when approximately ⅔ of the ascorbic acid was destroyed in 15 cases.

Ascorbic acid is probably not the only constituent related to the development of oxidized flavor. Since the theoretical ratio of oxygen/ascorbic acid disappearance is disturbed it is evident that other oxidizable constituents are involved. P.H.T.

637. The Bacterial Flora of Milk Pasteurized by the "In Bottle" Process. A. Rowlands, Midland Agr. Col., and A. L. Provan, Harper Adams Agr. Col. Proc. Soc. Agr. Bacteriologists (Eng.), 29–30. 1941.

Forty-two pint samples of milk were examined during storage at 15.5° C. The mean keeping quality was 2.82 days. Acid-producing organisms were predominant in fresh samples, but at the end of 48 hours were superseded by alkali-producing and peptonizing types, which formed only a relatively small proportion of the total flora of fresh samples. After 72 hours, organisms producing an acid clot reaction in litmus milk predominated.

M.W.Y.

638. Chlorine Sterilization in Practice and in the Laboratory. F. K. Neave and W. A. Hoy. Natl. Inst. Res. in Dairying, Univ. Reading. Proc. Soc. Agr. Bacteriologists (Eng.), 37–38. 1941.

Tinned trays were artificially infected with a 10% milk suspension of *Staphylococcus aureus* and dried at 37° C. for 1 hour before use. Chlorine solutions were poured on to the trays, which were mechanically agitated. The time taken for a reduction of 99.9% in the number of bacteria on trays rinsed with solutions containing 400, 200, 100 and 25 p.p.m. available chlorine was respectively 1, 1.3, 2.5, and 6.5 minutes. All tests were made with solutions prepared in tap water at a temperature of 55 to 60° F.

M.W.Y.

639. The Methylene Blue and Resazurin Tests for Pasteurized Milk. A. Rowlands, Midland Agr. Col., and A. L. Provan, Harper Adams Agr. Col. Proc. Soc. Agr. Bacteriologists (Eng.), 36. 1941.

The results from 160 samples showed that neither the methylene blue nor the resazurin test at 37° C. is a reliable measure either of recontamination or of the keeping quality of pasteurized milk when the samples are examined immediately after removal from cold storage on the morning after processing. Results of the methylene blue test at 15.5° C. indicate that some preliminary incubation is necessary before either of these tests is applied.

M.W.Y.

640. Bacteriological Indices of the Sanitary Quality of Market Cream. Elizabeth D. Robinton, Earle K. Borman, and Friend Lee Mickle, Bur. of Lab., Conn. State Dept. Health, Hartford, Conn. Amer. Jour. Pub. Health, *32*, No. 5: 464–470. 1942.

A total of 730 samples of raw, import and retail pasteurized cream were tested for the enumeration of bacteria by the following laboratory methods: 48 hours plate count at 37° C., at 55° C., and at 20° C., 4 days plate count at 8° C., and the direct microscopic clump count. The results indicate that the direct microscopic clump count is the most satisfactory and practical method for this purpose, especially if the sample, when pasteurized, is supplemented by a coliform determination and a phosphotase test. The direct microscopic method is the best method for detecting spore-forming organisms which ordinarily do not produce colonies on agar plates incubated at 8° C., 20° C., 37° C., and only rarely develop at 55° C.

M.W.Y.

641. San Francisco's Pasteurization Ordinance Finally Upheld. Editorial. Amer. Jour. Pub. Health, *32*, No. 5: 470. 1942.

An ordinance banning the sale of milk in San Francisco, except certified, unless it was pasteurized, which was passed in 1933 by the Board of Supervisors, was finally upheld as a proper exercise of the police power by a decision of the Supreme Court of California rendered April 2, 1942.

M.W.Y.

642. Milk Delivery Costs. ANONYMOUS. Milk Dealer, *31,* No. 8: 54. May, 1942.

A study made by the California Department of Agriculture showed that: When more than case lots are delivered at each wholesale stop, time studies revealed, the average delivery cost is 0.8383 of a cent per quart and in those instances where less than case lots are delivered the average wholesale delivery cost is 1.4604 cents, or a saving of 0.622 of a cent per quart. Study of costs pertaining to savings in multiple retail delivery sales reveal cost per single quart is 3.5758 cents, while the cost per quart in multiple sales is 2.7715 cents, or a saving of 0.8043 of a cent per quart.

Tables are presented showing "breakdown" information on wholesale and retail delivery costs. C.J.B.

643. "Vemp" Campaign. ANONYMOUS. Milk Dealer, *31,* No. 8: 33, 70. May, 1942.

A survey conducted by Milk Foundation, Inc., of Chicago, in which 1,552 housewives in Chicago, Cleveland, Indianapolis, Columbus, and Dayton revealed that 43% of the adults interviewed did not drink milk because they disliked it; 44% of the adult males and 49% of the adult females drank no milk at all; 9% of children 6 to 12 years of age and 13% of children 13 to 18 years of age drank no milk at all.

As a result of this survey a promotion campaign to sell more milk has been started. The campaign is built around "Vemp," a word made up of the first letters of the words "Vitamins," "Energy," "Minerals," and "Proteins."

The following steps for action are suggested: 1. Stress importance of adequate daily amounts of milk to tie in with present government campaign for adequate nutrition. 2. Direct campaign to adults because adults account for two-thirds of population and for 76% of milk deficiency below recommended minimums. 3. Direct campaign to both men and women because consumption deficiency is split nearly equally. 4. Feature milk drinking but keep it above beverage class by stressing nutritional superiority to meet beverage competition. 5. Play up food values. 6. Play up between-meal and bedtime drinking because this market is only partially developed and competition is weaker here than at meal time. 7. Meet the taste factor squarely, since taste loomed large in the survey as a reason for not drinking milk. 8. Play up the economy of milk. 9. Stress refreshing quality of milk in hot weather. 10. Tie into general nutrition-consciousness and government program. C.J.B.

644. Studies Relating to Soft Curd Milk. J. C. MARQUARDT, N. Y. Agr. Expt. Sta., Geneva, N. Y. Milk Dealer, *31,* No. 8: 27–28, 72. May, 1942.

A procedure for predicting soft curd values is described. Perfections have been made in gravitation studies involving 3-layer components based upon the creaming factor for normal milk. Composition and curd character in components of normal and homogenized milk and heat treatments to produce soft curd milk have been studied. The following conclusions are drawn: 1. A curd value in Hill units of 20 or less can be predicted when a value of 10 Hill units or less is attained after a two-minute setting period at 90° F. 2. Three-layer component gravitations have advantages over two-layer separations in measuring homogenization efficiency. 3. The fat, solids and ash are evenly distributed in various components of gravitated homogenized milk. This is not true with unhomogenized milk. 4. The curd strength of gravitated homogenized milk components is very uniform. This is not true with unhomogenized milk. 5. It is possible by employing certain heat treatments to produce milk that has a curd strength comparable to that of properly homogenized milk. 6. Homogenization decreases the normal setting time of milk. C.J.B.

PHYSIOLOGY

645. Factor of Age in the Rate of Absorption of, and in Mammary Stimulation by, Testosterone Monopropionate Pellets in Rats.

Thomas R. Forbes, John Hopkins Univ., Endocrinology, *30*, No. 5: 765. May, 1942.

Testosterone monopropionate pellets weighing 8.4 mg. to 10.5 mg. were implanted in 4 male and 5 female litter-mate rats aged 16 days; in 4 males and 5 females, aged 47 and 54 days; and in 5 males and 4 females, aged 12 months. Each rat received one pellet and gross observations were made of the mammary glands 12 days after implantation. Neither males nor females of the 16-day-old group showed any gross mammary development. Three males and 2 females of the 47- and 54-day-old group showed no appreciable mammary stimulation. The mammary glands of one male and 3 females showed slight stimulation. The mammary glands of all 5 males and 2 females of the 12-month-old group were moderately developed and the other 2 females showed marked mammary growth. R.P.R.

646. Effects of Estrogens upon the Young of Injected Lactating Rats.

Charles K. Weichert and Sylvia Kerrigan, Univ. Cincinnati. Endocrinology, *30*, No. 5: 742. May, 1942.

The litters of each of 15 rats were reduced to 3 males and 3 females and the mother rats injected with an estrogen immediately after parturition. The weights of each mother and litter were recorded daily. The young grew normally for 5 or 6 days and then failed to gain weight at the normal rate.

On the 14th day the weights of the young of injected mothers ranged from 11 to 17 gm. as compared with an average weight of 26.5 gm. for those of untreated controls. Lactation did not seem to be suppressed inasmuch as milk exuded from the cut surface of the mammary glands at autopsy and sections showed alveoli distended with secretion. Milk was also present in the stomachs of most of the young when they were autopsied. It was thought that loss of maternal instinct probably accounted in part for failure of the young to grow normally. R.P.R.

647. Studies Concerning the Mechanism Controlling the Initiation of Lactation at Parturition. I. Can Estrogen Suppress the Lactogenic Hormone of the Pituitary? JOSEPH MEITES AND C. W. TURNER, Univ. Mo. Endocrinology, *30,* No. 5: 711. May, 1942.

The daily administration of 2 mg. of either diethylstilbestrol or testosterone propionate to lactating rats for the first 6 days postpartum increased the pituitary lactogen content, caused some reduction in the amount of milk present in the mammary glands, and resulted in the death of 38 and 11% respectively of the young rats. Pituitaries from lactating rats which were not suckled for the first week after parturition contained 50% less lactogen than suckled rats and the mammary glands from these rats were practically devoid of milk. The injection of 0.0125 to 100 mg. of diethylstilbestrol over a 5-day period into normal immature and mature male guinea pigs increased the lactogen content of their pituitary glands up to 438%, the smaller dosages being more effective than the larger dosages. R.P.R.

648. Studies Concerning the Mechanism Controlling the Initiation of Lactation at Parturition. II. Why Lactation is not Initiated During Pregnancy. JOSEPH MEITES AND C. W. TURNER, Univ. Mo. Endocrinology, *30,* No. 5: 719. May, 1942.

Immature female guinea pigs weighing approximately 300 gm. were injected with either one mg. of progesterone, 25 to 200 International Units of estrone, or one mg. of progesterone plus 25 to 200 I.U. of estrone daily for 5 days. Progesterone alone had no effect on the lactogen content of the pituitary, estrone alone caused definite increases in pituitary lactogen content, and certain combinations of progesterone and estrone either entirely prevented or reduced the increase in pituitary lactogen content which was obtained with estrone alone. It was suggested that during pregnancy the progesterone-estrogen ratio is such that progesterone overrides the lactogen-stimulating effects of estrogen and that this action accounts for the fact that the pituitary lactogen content remains as low during pregnancy as in the nonpregnant state. R.P.R.

649. Studies Concerning the Mechanism Controlling the Initiation of Lactation at Parturition. III. Can Estrogen Account for the Precipitous Increase in the Lactogen Content of the Pituitary Following Parturition? JOSEPH MEITES AND C. W. TURNER, Univ. Mo. Endocrinology, *30,* No. 5: 726. May, 1942.

The effect of the length of estrogen treatment on pituitary lactogen content was determined in 40 male guinea pigs. Eleven similar pigs receiving no treatment served as controls. All experimental pigs received the same dosage of estrone (3000 I.U.) over periods of time varying from one to 30 days. A 142 and 204% increase in pituitary lactogen content was obtained in the groups injected for 1 and 2 days respectively and a 371% increase was secured in animals injected for 5 days. The animals injected for 10 to 30 days did not show a significantly greater increase over those injected for 5 days. Four New Zealand White rabbits were hysterectomized on the 20th day of pregnancy and they were killed 5 days later. Individual assays of the pituitaries showed that an average increase of 83% in pituitary lactogen content had been obtained. R.P.R.

650. Progesterone-Like Activity of Some Steroid Compounds and of Diethylstilbestrol in Stimulating Mammary Lobule-Alveolar Growth. JOHN P. MIXNER AND CHARLES W. TURNER, Univ. Mo. Endocrinology, *30,* No. 5: 706. May, 1942.

Various compounds were assayed for their mammary lobule-alveolar growth promoting properties by injecting them simultaneously with a standard dosage of estrone into ovariectomized virgin mice. The criterion of response was the percentage of mice showing a minimum amount of lobule-alveolar growth after 10 daily subcutaneous injections. Employing progesterone as a standard, pregneninolone had an activity of approximately one-half, desoxycorticosterone acetate and dehydroandrosterone of one-third, diethylstilbestrol of one-fourth, acetoxy-pregnenolone of one-sixteenth, and methyl testosterone of one twenty-fifth, that of progesterone. Testosterone and testosterone propionate in dosages up to 10 mg. failed to stimulate lobule-alveolar growth. R.P.R.

651. Effect of the Gonadotropic Substance of Pregnant Mare's Serum on the Blood Plasma-Ascorbic Acid of the Castrate Bovine. FREDERICK N. ANDREWS AND RALPH E. ERB, Purdue Univ. Endocrinology, *30,* No. 5: 671. May, 1942.

The injection of 1000 to 2000 rat units of pregnant mare's serum into grade Hereford steers approximately two years of age was followed by decreases in venous blood plasma-ascorbic acid of 42 and 56% respectively within a period of 5 hours. The recovery of blood-plasma-ascorbic acid to approximately pre-injection levels required 44 to 68 hours. R.P.R.

652. Progesterone Effect on Pituitary Lactogen Content and on Mammary Glands of Ovariectomized Rats. R. P. Reece and J. A. Bivins, N. J. Agr. Expt. Sta. Soc. Expt. Biol. and Med. Proc., *49,* No. 4: 582. Apr., 1942.

The daily injection of 15 mg. of progesterone for 10 days into sexually mature spayed rats increased the lactogen content of their pituitary glands, caused no significant increase in pituitary weight, and induced moderate development of the lobule-alveolar system and limited secretory activity of the mammary glands. The daily administration of 15 mg. of progesterone plus 33 micrograms of estrogen for 10 days caused extensive lobule-alveolar growth of the mammary glands. The secretory activity of the glands was somewhat greater than that following progesterone treatment. This treatment was less effective than estrogen but more effective than progesterone in increasing the lactogen content of the pituitary gland. R.P.R.

653. Lobule-Alveolar Growth of Mammary Glands of Hypophysectomized Female Rats. Ralph P. Reece and Samuel L. Leonard, N. J. Agr. Expt. Sta. and Cornell Univ. Soc. Expt. Biol. and Med. Proc., *49,* No. 4: 660. Apr., 1942.

Twenty sexually mature virgin rats were ovariectomized and hypophysectomized. Beginning on the day following operation 6 of the rats were injected subcutaneously daily for 15 days with 300 micrograms of testosterone propionate and the remaining 14 rats received subcutaneously 300 micrograms of testosterone propionate plus 0.5 cc. of growth hormone intraperitoneally daily for 15 days. The rats were sacrificed on the day after the last injection and the right abdominal mammary glands studied as whole mounts. The mammary glands of the testosterone-treated rats showed involutionary changes but the lobule-alveolar system of the mammary glands was developed in 12 out of 14 rats receiving the combined treatment.

R.P.R.

654. Assay of Adrenals for Lactogenic Hormone. V. Hurst, J. Meites and C. W. Turner, Univ. Mo. Soc. Expt. Biol. and Med. Proc., *49,* No. 4: 592. Apr., 1942.

Two commercial adrenal cortical extracts, whole untreated rabbit adrenals, and isoelectric precipitates of beef, hog and rabbit adrenals were assayed for lactogenic hormone by the intradermal pigeon crop gland method. None of the adrenal compounds was found to contain the lactogenic hormone. R.P.R.

655. Effects of Testosterone Propionate in Spayed Female Rats. G. L. Laqueur, Stanford Univ. Soc. Expt. Biol. and Med. Proc., *49,* No. 3: 425. Mar., 1942.

Six rats were spayed during estrus and 6 rats were spayed on the second day of diestrus. 4 mg. of testosterone propionate were administered subcutaneously every other day over a period of 20 days. The animals were killed 24 hours after the last injection. The mammary glands showed an increase of interlobular fibrous tissue, overgrowth of the ducts, some evidence of secretion but no proliferation of the alveoli. R.P.R.

656. Effect of Certain Hormones and Drugs on the Perfused Mammary Gland.

W. E. PETERSEN, University of Minnesota. Soc. Exp. Biol. and Med. Proc., *50,* No. 2: 298. June, 1942.

The experiments were carried out on bovine mammary glands perfused according to a previously reported technic. Glands were obtained from a packing plant and the teats were cannulated to permit drainage of the milk immediately after the perfusion started and the cannula left in situ during the experiment. Blood pressure was maintained at 110 mg. Hg pressure and the tested substances were injected into the arterial blood as it entered the gland. Pitocin injections were made following the injection of other substances as a test for the completeness of the milk ejection. Pitocin and acetylcholine caused complete ejection of milk, the former caused a decrease in blood flow while the latter had no influence on blood flow. Pitressin caused an incomplete ejection of milk and a marked decrease in blood flow. Epinephrin and histamine caused a partial ejection of milk and a marked decrease in blood flow. Ergonovine had no effect on milk ejection but caused some vasoconstriction. Acetyl-B-methylcholine caused a partial ejection of milk and carbamylcholine had no effect on milk ejection. Atropine slightly increased the blood flow and completely prevented any response to acetylcholine and acetyl-B-methylcholine but had no influence on the action of pitocin or pitressin. The results were believed to indicate that the mammary gland is innervated by the parasympathetics as well as the sympathetics. R.P.R.

657. Forced Ovulation of Normal Ovarian Follicles in the Domestic Fowl.

R. M. FRAPS, M. W. OLSEN AND B. H. NEHER, U.S.D.A. Soc. Exp. Biol and Med. Proc., *50,* No. 2: 308. June, 1942.

Ovulation of the normally growing follicle of the hen's ovary was induced prematurely by as much as 17 hours by the intravenous injection of several hormones. A luteinizing principle from horse anterior pituitaries caused ovulation of follicles 10 to 11 hours prior to time of normally expected ovulation in 100% of injected hens. Prehysin, Gonadin, and Anteron were effective when injected at sufficiently high levels. At certain submaximal injection levels the percentage of ovulating hens decreased with increasing prematurity of the ovarian follicles and this effect was more pronounced in follicles of relatively great prematurity at the time of effect of the injected hormone. R.P.R.

658. Time Required for Induction of Ovulation Following Intravenous Injection of Hormone Preparations in Fowl. R. M. Fraps, G. G. M. Riley and M. W. Olsen, U.S.D.A. Soc. Exp. Biol. and Med. Proc., *50*, No. 2: 313. ' June, 1942.

Ovulation time following intravenous injection of gonadotropic hormones into pretreated and normally laying hens was determined. The interval in pretreated hens averaging more than 2 ovulations per hen at time of autopsy was 6.8 hours. Ninety per cent of 69 normally laying hens ovulated from 6.5 to 8.5 hours following injection and the remaining 10% required up to 9.6 hours. The minimal ovulating interval for pretreated hens was 6.1 hours and for non-pretreated hens, 6.5 hours. R.P.R.

659. Functions of the Adrenal Cortex. Frank A. Hartmen, Ohio State University. Endocrinology, *30*, No. 6: 861. June, 1942.

Cortilactin, an extract of the adrenal cortex, was recently prepared by isoelectric precipitation. This factor enables lactating rats kept in good condition by other adrenal fractions to lactate adequately so that all of their pups grow at a normal rate. Cortilactin does not affect glyconeogenesis and full replacement has been obtained by means of Kendall's compound E. The author suggests that it seems quite possible that more than one factor may be effective in lactation. R.P.R.

660. Mammogen and Unilateral Mammary Gland Growth in the Rabbit. A. A. Lewis and C. W. Turner, University of Missouri. Endocrinology, *30*, No. 6: 985. June, 1942.

Unilateral percutaneous treatment of male rabbits with estrone or diethylstilbestrol caused either greater mammary gland development on the treated side or development of treated glands only. In 2 of 3 rabbits the injection of estrone and the unilateral percutaneous application of turpentine caused the development of larger mammary glands on the turpentine-treated side. Turpentine-treated mammary glands from diethylstilbestrol-injected rabbits, however, did not show any increased size over that of untreated glands. One rabbit treated unilaterally percutaneously with turpentine had several mammary glands showing some duct development on the treated side but 3 additional rabbits gave negative results. Other irritants, oil of myrrh, medical turpentine, oil of cantherides, and eucalyptus oil, applied unilaterally to the skin gave negative results. R.P.R.

661. Pituitary Weight in Growing New Zealand White Rabbits in Relation to Live Weight. H. H. Kibler, A. J. Bergman and C. W.

TURNER, University of Missouri. Endocrinology, *31*, No. 1: 59. July, 1942.

Pituitary and associated body weights for growing New Zealand White rabbits were presented in a logarithmic chart. A covariance analysis of pituitary to body weight relations established a statistically significant difference due to sex. The ratio of pituitary to body weight was found to decline with increase of body weight during growth. Fitted equations relating pituitary and body weights were compared with curves for mature animals of different species for a similar range of body weights. Prediction tables of pituitary weight from body weight and for proportioning pituitary or metabolism stimulating injections according to pituitary weight were presented. R.P.R.

662. Influence of Some Steroid Hormones on Lactation in Adrenalectomized Rats. ROBERT GAUNT, W. J. EVERSOLE AND E. C. KENDALL, New York University and Mayo Foundation. Endocrinology, *31*, No. 1: 84. July, 1942.

The influence of various steroids of the adrenal cortex and of the gonads on the deficient lactation of adrenalectomized rats was studied. Indications were that all substances known to relieve adrenal insufficiency were somewhat helpful. Desoxycorticosterone acetate gave variable results as it was helpful in some but not in all rats and in no case maintained lactation equal to the average control figure. Normal lactation for the first 17 days was obtained with compound E and the whole extract of the adrenal cortex. A tentative conclusion was drawn that full mammary secretion is dependent on those adrenal factors which affect primarily carbohydrate metabolism. Estrogen and androgen inhibited lactation even in small doses but progesterone did not inhibit lactation. R.P.R.

663. Non-Effect of Hysterectomy upon the Mammary Gland of the Monkey. HAROLD SPEERT, Carnegie Institution of Washington and Johns Hopkins Hospital. Endocrinology, *31*, No. 1: 97. July, 1942.

Hysterectomy was performed on an adult rhesus monkey on day 22 of an ovulatory menstrual cycle and observed over a period of 6 months. Hysterectomy failed to delay involution of the corpus luteum and had no effect on the mammary glands. R.P.R.

664. Mammary Growth in Male Mice Fed Desiccated Thyroid. W. U. GARDNER, Yale University. Endocrinology, *31*, No. 1: 124. July, 1942.

The mammary glands of 13 out of 14 intact male mice fed desiccated thyroid mixed with their feed, 1.5 mg. per kg., showed proliferation of the ducts and hyperplastic end-buds. Similarly treated castrated male mice showed no mammary growth. The adrenal glands of the thyroid-fed intact or castrated male mice were greatly increased in weight and the kidneys and livers were also larger than those of the controls. The amounts of desiccated thyroid consumed were insufficient to reduce the gain in body weight below that of the controls. R.P.R.

655. Effects of Lactogen on Normal and Adrenalectomized Female Rats.

Charles E. Tobin, University of Rochester. Endocrinology, *31*, No. 2: 197. Aug., 1942.

Daily subcutaneous injections of lactogen for a period of 4 to 10 days to normal rats caused maintenance of corpora lutea which apparently secreted progestin. Bilaterally adrenalectomized rats similarly treated from the day of operation until death or until the 15th day for those which survived longer had a significantly prolonged survival time when treated with one lactogen (Difco) but not with the other one (Schering) and showed better maintenance of follicles and corpora lutea than untreated controls.

R.P.R.

666. Relation of Thyroid to Growth. I. Effects of Crystalline Thyroxin upon Rate of Growth, Food Intake and Body Composition of Female Albino Mice.

Marvin Koger, Victor Hurst and C. W. Turner, University of Missouri. Endocrinology, *31*, No. 2: 237. Aug., 1942.

Virgin mice weighing from 13 to 16 gm. were injected subcutaneously daily with crystalline thyroxin in doses ranging from 0.015 to 0.04 mg. The thyroxin-treated animals repeatedly gained an average of 28% more weight during a 5-week period than did the controls. The maximum difference was attained in about 5 weeks after which time the difference gradually became less but the body weight of the thyroxin-treated mice did not exceed that which was finally attained by the controls. The daily food intake of the thyroxin-treated mice was approximately 25% above that of the controls. The carcasses of the injected mice had a higher percentage of water and nitrogen and a lower percentage of fat than did those of the controls. Thyroxin-injected mice stored more nitrogen and gained more in body weight per unit of food intake than did controls for the first few weeks of treatment. After the rapid gains had subsided the controls were more efficient than were treated mice. Control mice stored more fat and more energy per unit of food intake than did the thyroxin-injected animals. The practical application of the results to the livestock industry was discussed.

R.P.R.

667. Pituitary Weight of Growing Male Albino Rat Related to Body

Weight. JOHN P. MIXNER AND CHARLES W. TURNER, University of Missouri. Endocrinology, *31,* No. 2: 261. Aug., 1942.

One hundred and thirty-five male albino rats of the Wistar strain were used to obtain data relating pituitary and body weights. Data were correlated by fitting the data by the product moment method to the equation $Y = a + bX$ and by fitting the points on the line of means by the method of least squares to the equation $Y = aX^b$. The latter equation indicated that pituitary weight was not directly proportional to body weight but varied with the 0.68 power of body weight. R.P.R.

668. Reconsideration of the Significance of Some Plant Characteristics in Relation to Yield of Gonadotropic Material. JOHN W. MITCHELL, RUBIN BORASKY AND JAMES T. BRADBURY, U.S.D.A. Endocrinology, *31,* No. 2: 283. Aug., 1942.

Data collected during the last 3 years showed that ovulation was induced in rabbits as the result of the administration of some plant juice extracts during the spring and summer months as measured by the arbitrary standards previously described. Many negative results were observed during all seasons of the year. Positive assays were not found to be correlated with any of the plant characteristics noted such as age, succulency, hydrogen-ion concentration of the juice, or with any factor of the environments under which the plants were grown. R.P.R.

MISCELLANEOUS

669. The Application of Plate Type Evaporators to Truck Bodies. ALBERT F. SAWYER, Development Engineer, Dole Refrigerating Co., Chicago, Ill. Refrig. Engin., *43,* No. 6: 349. June, 1942.

Vacuum plates consist of two sheets of metal forming a flat box within which are evaporative coils of steel tubing. A vacuum is drawn on the space between the metal sheets which draws them against the tubing. Edges and corners are welded to form a hermetically sealed compartment. If holdover capacity is desired, a eutectic solution is placed in the inter-plate space before exhausting. Two types of holdover plates are made, those of comparatively thin cross section for 4- to 5-hour holdover effect, and others of 2⅝-inch thickness for 12- to 18-hour holding. These latter require spacers over the coils to build up to the required thickness.

Eighteen-degree solution is recommended for temperatures of 36° F., while − 6° solution is required for temperatures of 0° to 5° F. K factor for the plates is set at 2 B.t.u., and a temperature difference of 18° for the 36° F. work, while a t.d. of 15° is selected for the low temperature duty involved in ice cream trucks or trucks handling low temperature frozen foods. This is because of the inherent refrigeration in frozen foods and ice cream, the latter loading in at - 10° F. or lower.

Low temperature holdover plates may be located in practically any position with satisfactory results, while high temperature plates are best located on side walls with drip trough placed below them. Long distance transportation trucks must be equipped with refrigerating units powered either by separate power plants or power take-offs, and the system designed to operate about 18 hours out of 24, using either dry plates or partial holdover plates. L.M.D.

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ABSTRACTS OF LITERATURE

BOOK REVIEW

670. Nutrition and Chemical Growth in Childhood, Volume I: Evaluation. ICIE G. MACY, Director of Research Laboratory of the Children's Fund of Michigan. Charles C. Thomas, Publisher, Springfield, Illinois.

The purpose of this book is not especially to trace new scientific problems but as stated, "to contribute to the enlargement of scientific knowledge of nutrition and growth in childhood." This means, that even though the results might only confirm earlier experiences, its purpose still will be fulfilled. The value of the work presented here lies in its fine organization, the thoroughness with which the experiments have been carried out and the honest and well-arranged presentation, which altogether very well might serve as a pattern for other similar investigations.

The book is divided in two main parts. The first part presents results from the ten-year study, in which 50 physicians, chemists, dieticians and home-economists have participated. The last part outlines the chemical methods used by the authors. It is possible to follow the planning of the experiments and the experiments themselves in all details.

The great majority of the numerous observations and figures presented are original and only now and then compared with other investigators' results, when such comparison was found proper for a verification. This fact increases the value of the book considerably and makes it a precious source of information for any nutritionist or pediatrician when planning diets for children.

The "Introduction" and "General Considerations" deserve special attention. Some of the statements here are of fundamental character as, *i.e.*, "The purpose of the intensive investigations of normal children, which have been carried out in this laboratory for ten years, has been to obtain sufficient knowledge of the normal child to enable comparative evaluation of the metabolism of the ill child."

No doubt, Dr. Macy touches here something very important. Too often our attention is attracted one-sidedly to the sick child, but for a sound judgment we cannot very well know too much about the food metabolism of the healthy child, and here Dr. Macy's book furnishes ample contributions to earlier knowledge. The justification for the investigation of this, the normal child's nutrition and growth, is expressed in another statement: "It is evident, that the attainment of maximum capacity toward maturity requests growth and development during optimal nutritional conditions."

Finally it is worth while to point out that the experiments presented show that the best food science at present is able to give children apparently does not always procure sound and strong teeth without caries. There is evidently still some possibility for further improvement in children's nutrition, especially in regard to the mineral metabolism.

The second part of the investigation of Dr. Macy and coworkers, called "Volume II: Interpretation," in which the implications will be discussed, must be looked for with keen interest. Bernard Spur.

BREEDING

671. The Effect of the Age at the First Calving on Growth and Fat Production and the Results with Respect to Economy of Production. Åldern vid första kalvduktionsresultatet.)

Artur Hansson, Lantbrukshögskolans institution för avelsochraslära. Kungl. Lantbruksakademiens Tidskrift, *80*, No. 5: 387–412 (English summary, 411–412). 1941.

The effect of the age at the first calving on the growth and fat production of Swedish Red and White Cattle (S.R.B.) was studied. The growth of the cow continues until it is 6 years of age. The growth is retarded by the milk secretion during the first lactation, otherwise the full-grown stage would probably be reached at 4½ to 5 years of age. The age at first calving has no effect on the ultimate size of the cow. This conclusion, however, is based only on heifers which have reached at least 27 months of age before calving. The butter-fat yield during the first lactation increases with increasing age at first calving. After the cow has reached 36 months of age, the rise in yield with increasing age is very slight. This is probably due to the fact that more than one gestation is needed for a full development of the udder of the cow. The fat production of the mature cow (7-9 years old) is not influenced by her age at first calving. The age at first calving has no effect on the fat content on the milk. The lifetime record of the cow shows a tendency to decrease, and the age at culling to increase with increasing age at first calving. The latter depends on a more intensive selection of young first calvers. The total fat production until 8 years of age decreases greatly with increasing age at first calving. A first-calving interval of 13 to 15 months and a second of not more than 13 months results in the greatest daily fat production during these two intervals. Cows calving the first time at a low age should not be bred again before 4–5 months after calving. The relation between the cow's production of butter-fat and her feed consumption during her whole life, viz., the economy of the milk production, is greatly influenced by the age at first calving. If the average age of the cows at first calving is reduced by 6 months, the fat production per 1000 feed units increases by 3.5 kg. butter-fat. It may be concluded that the most suitable age at first calving for cows of the Swedish Red and White breed is about 26–28 months. R.E.L.B.

672. The Influence of Inbreeding on Birth Weight, Rate of Growth, and Type of Dairy Cattle. J. W. BARTLETT, R. P. REESE, AND O. L. LEPARD, N. J. Agr. Expt. Sta., New Brunswick, N. J. Jour. Anim. Sci., *1*, No. 3: 206–212. Aug., 1942.

This is a progress report of a long-time project in which 45 Holstein-Friesian cows were selected as foundation animals on the basis of size, good type, and mature equivalent Class C fat production of 480 pounds with the milk testing at least 3.6% butterfat. Four bulls were selected as foundation sires at the beginning of the experiment. The progeny of the foundation animals were mated as follows: (1) sire-daughter matings; (2) brother-sister matings; (3) matings with less than 50% of the same blood and (4) outbreeding. The last system of mating was used as a control.

Birth weights and measurements on 76 inbred and 76 outbred heifers show that the inbred heifers were as large at birth and grew as rapidly as the outbred animals. There appeared to be no relation between the intensity of inbreeding and type. C.F.H.

673. Effect of Diluters and Storage upon Fecundity of Bovine Semen. G. K. L. UNDERBJERG, H. P. DAVIS, AND R. E. SPANGLER, Univ. Nebr., Lincoln. Jour. Anim. Sci., *1*, No. 2: 149–154. May, 1942.

Fresh semen samples which had the following treatments were used to inseminate a total of 931 cows: (1) no treatment; (2) diluted with fresh egg yolk buffer; (3) diluted with stored egg yolk buffer; (4) diluted with autoclaved milk. A corresponding set of samples with the same treatments were stored for varying lengths of time prior to insemination.

The treatments accorded the fresh and stored semen in the various groups had no significant effect on the percentages of conception when compared with the controls. Although the semen samples which were stored had a high degree of motility of the spermatozoa, the conception percentages were significantly lower. The authors suggest that other unknown factors are in operation which cause loss of the fertilizing capacity of spermatozoa despite the retention of a high degree of motility. C.F.H.

BUTTER

674. Flavor Development in Salted Butter by Pure Cultures of Bacteria. W. H. HOECKER AND B. W. HAMMER, Iowa Agr. Expt. Sta., Ames, Iowa. Iowa Agr. Expt. Sta. Res. Bul. *290*. Aug., 1941.

Pure cultures of various streptococci produced relatively large amounts of diacetyl and acetylmethylcarbinol in milk containing added citric acid. These included *S. citrovorus* or *S. paracitrovorus, S. diacetilactis, S. citrophilus* and an unidentified organism H28. *S. aromaticus,* which does not ferment citric acid, produced diacetyl and small amounts of acetylmethylcarbinol in milk. With each of the species the ratios of diacetyl to acetylmethylcarbinol varied in the different trials; frequently, the diacetyl was much higher in proportion to the acetylmethylcarbinol than with butter cultures.

The diacetyl contents of cream-plus-culture immediately after mixing were both higher and lower than the theoretical amounts calculated from the diacetyl contents of the cream and culture; the carbinol contents were about the same as the theoretical values in most trials, but were higher in some instances.

In cream-plus-culture held 16 hours at approximately 40° F., the diacetyl and acetylmethylcarbinol contents increased when butter culture, *S. diacetilactis* or *S. citrophilus* was used; decreases often occurred with *S. citrovorus* or *S. paracitrovorus;* usually, little or no change occurred with organism H28 or *S. aromaticus.*

Butter made without the use of culture or with *S. aromaticus* contained only small amounts of diacetyl and acetylmethylcarbinol, whereas butter made with the other cultures commonly contained appreciable amounts of these compounds.

Only small percentages of the diacetyl and acetylmethylcarbinol present in cream-plus-culture were retained in the butter, the remainder being in the buttermilk; the percentage retention was essentially the same with the different cultures, although with each culture there was considerable variation from one churning to another

Both increases and decreases in diacetyl occurred in butter held 1 day at 40° F. and then 2 and 4 weeks at 35° or 0° F., the larger changes usually occurring with butter held at 35° F. Occasionally, increases in diacetyl contents after 2 weeks were followed by decreases after 4 weeks. Except in a few instances, the acetylmethylcarbinol contents did not change appreciably.

Butter made with butter culture, *S. citrovorus, S. paracitrovorus* or *S. citrophilus* usually contained relatively large amounts of diacetyl and acetylmethylcarbinol and commonly placed high in a series of churnings. However, butter containing exceptionally large amounts of diacetyl and acetylmethylcarbinol sometimes placed low and was criticized as being coarse, sour, oily or containing some other objectionable flavor. Butter made without culture or with *S. aromaticus* contained only small amounts of diacetyl and acetylmethylcarbinol and usually placed low. Author's Abstract.

CHEESE

675. Calcium Oxide and Sugar in Cheese Manufacture. J. C. Marquardt, N. Y. Agr. Expt. Sta., Geneva. Natl. Butter and Cheese Jour., *33*, No. 8 : 40. August, 1942.

After preliminary laboratory tests, ten batches of cheese were made in a New York State cheese factory during April, 1941. The "fodder milk" delivered on 4 days represented the regular make; 2 oz. calcium oxide and 12 oz. of sugar were added to each 1000 lbs. of milk on each of three days; on three days half of these amounts of oxide and sugar were added to the milk and half to the curd. After 6 months curing at 40° F. the cheese were scored. Two of the four controls were called "good" while two were criticized for fodder flavor. When the oxide and sugar were added to both milk and curd there was "slight checking in the cheese texture." The three lots of cheese made with all the oxide and sugar in the milk were "good," two were like grass cheese, the third had cheese flavor. Further holding for 30 days at room temperature and 60 days cold storage confirmed the beneficial effects. The author states that the calcium oxide is transformed to calcium lactate which is less astringent than lactic acid, thus rendering less pronounced such undesirable flavors as fodder, unclean and others. The action of sugar is difficult to explain. W.V.P.

676. Wisconsin Cheese. Walter V. Price and Catherine J. Personius, Depts. Dairy Inds. and Food Technol. Res., Univ. Wis. Wis. Med. Jour., *41*, No. 2: 126–128, 170. 1942.

R.E.L.B.

CHEMISTRY

677. Some Aspects of the Rate of Reaction of Oleic Acid with Oxygen. J. L. Henderson and H. A. Young, Univ. Calif., Davis. Jour. Phys. Chem., *46*, No. 6: 670. 1942.

Purified oleic acid, and preparations (three methods of purification cited) was introduced into reaction chambers, and under controlled conditions subjected to oxygen systems. The rate of oxygen absorption was determined by use of iodine and peroxide numbers tests. The rate of O_2 absorption was found to be a function of the concentration of oleic acid. Fivefold variation in oxygen pressure did not affect the induction period; when 0.1 mole of oxygen was absorbed per mole of acid, the oxygen absorption was then found a function of oxygen pressure and for which a rate equation was derived. Substantiative evidence is presented based on the formation of peroxides and the destruction of double bonds that peroxide formation is the first reaction in the oxidation of oleic acid. K.G.W.

CONCENTRATED AND DRY MILK: BY-PRODUCTS

678. Keeping Quality of Powdered Whole Milk. H. A. Hollender and P. H. Tracy, Univ. Ill., Urbana. Natl. Butter and Cheese Jour., *33*, No. 8: 8. Aug., 1942.

See Journal of Dairy Science, **25**, No. 3: 249–274. Mar., 1942.

W.V.P.

DISEASE

679. **Immunization Against Bovine Trypanosomiasis.** H. E. HORNBY. Trans. Roy. Soc. Trop. Med. and Hyg., *35,* No. 3: 165–176. 1941.

The experiment indicated that there is as yet no practical method of immunizing cattle against trypanosomiasis. Through Trop. Diseases Bul., *39,* No. 4: 242–244. 1942. R.E.L.B.

680. **Undulant Fever.** D. G. MAHLE, Plainview, Minn. Minn. Med., *25,* No. 3: 177–180. 1942.

A brief history of the origin and distribution of undulant fever is given with particular emphasis on Minnesota. The symptoms and characteristics of the disease are briefly described. Four personally observed and treated cases are reported with cures and no recurrences to date. Bang's testing of all cattle and pasteurization or boiling of all milk are essential in the control of this disease. R.E.L.B.

681. **New Developments in the Diagnosis and Treatment of Brucellosis (Undulant Fever).** WALTER M. SIMPSON, Kettering Inst. Med Res., Miami Valley Hospital, Dayton, Ohio. Minnesota Med., *24,* No. 9: 725–738. 1941.

An address. The most important consideration in the control of brucellosis is adequate, controlled pasteurization of all milk and other dairy products. R.E.L.B.

682. **A Milk-Borne Outbreak of Gastroenteritis in Oklahoma City.** IRVING M. TERZICH, U. S. Pub. Health Service, County-City Health Dept., Lawton, Okla. South. Med. Jour., *35,* No. 8: 773–780. 1942.

An outbreak of food poisoning affecting 71 individuals occurred in Oklahoma City and surrounding Oklahoma County communities on July 1–2, 1941. The cause of the outbreak is believed to have been a highly contaminated milk supply. The milk had not been properly pasteurized; hemolytic staphylococci and toxin-producing cocci were shown to be present as were organisms of the coli-aerogenes group. A large amount of neutralizer in the form of carbonates had been added. The victims of the food poisoning suffered from extremely acute gastroenteritis but no deaths were reported. The plant from which the suspected milk supply was obtained employed inadequate pasteurization equipment for the processing of that portion of the milk shipped to Oklahoma City and vicinity. The subsequent contamination of the milk is believed to have been due to an employee afflicted with skin eruptions on his arms, hands, and face, who capped the gallon jug containers by hand. The attacks of gastroenteritis were probably due to the ingestion of toxin-producing cocci and hemolytic staphylococci. R.E.L.B.

683. Undulant Fever. FREDERIC W. LATHROP, 909 Park Ave., Plainfield, N. J., Jour. Med. Soc. N. J., *37*, No. 9: 466–469. 1940.

The symptoms of Brucellosis in man are extremely variable, but particularly they are continued or recurring fever, fatigue, profuse sweats, loss in weight and neuritis. The treatment is best carried on by the use of sulfanilamide and brucellin therapy. The disease can be eradicated by pasteurization of all forms of milk and milk products, and eventually by the elimination of the infection in cattle and goats. R.E.L.B.

684. Acute Gastroenteritis Due to the Elaboration of Enterotoxin by *Staphylococcus aureus* in Buttermilk. O. L. CHASON AND C. H. WAITE, Mobile County Health Dept. and Bur. Lab. Ala. State Dept. Health, Mobile. Jour. Med. Assoc. State Ala., *11*, No. 11: 390-391. 1942.

Epidemiologic investigation of an outbreak of gastroenteritis indicated that it was attributable to the ingestion of homemade buttermilk. Eleven persons in 4 families became acutely ill after drinking the buttermilk. Six persons in 2 of the same familes, who drank no buttermilk but otherwise partook of the same foods, did not become ill. In a third family the buttermilk was used in the preparation of biscuits; one person complained of indigestion following the meal at which these biscuits were served. From the buttermilk a pure culture of *Staphylococcus aureus* was isolated, which, when grown under proper conditions, produced an enterotoxin demonstrable by the Dolman kitten test. R.E.L.B.

685. Undulant Fever. RAGNAR T. WESTMAN, U. S. Public Health Service, Kansas City. Jour. Kans. Med. Soc., *42*, No. 11: 468–71. 1941.

Undulant fever, due to raw milk, is an important disease in Kansas. The problem of prevention is discussed. Testing cows gives only poor protection, since the disease may appear in the herd and be transmitted through the raw milk between tests. No vaccination of cows offers any protection against undulant fever. Pasteurization affords the best safeguard against all milk-borne diseases, including undulant fever. R.E.L.B.

686. The Types of Tubercle Bacilli Isolated from Human Tuberculosis in Japan. KAORU URABE, Inst. Bact. Sitzber freien ärztl. Ver. med. Fakultät kaiserl. Kyûsyû-Univ. Hukuoka (276 Sitzung, 23 Februar 1940); Hukuka Acta Med., *33*, No. 7: 869 (in Japanese) (in English, 62). 1940.

Human tuberculosis of bovine origin is proportionately less common in Japan than in America and Europe. This may be due to the low percentage of tuberculous cattle in Japan and to the national custom of not drinking raw milk. R.E.L.B.

687. Internal Parasites of Cattle. G. DIKMANS. U. S. Dept. Agr. Circ. 614. Jan., 1942.

A comprehensive treatise on the subject indicated. Gives location, appearance, life history, distribution, symptoms, lesions, prevention and treatment of the important internal parasites, including roundworms, hookworms, lungworms, tapeworms, flukes, protozoans, and numerous others. J.G.A.

688. Bloat in Cattle. H. H. COLE, S. W. MEAD, AND M. KLEIBER. Calif. Agr. Expt. Sta. Bul. 662. Feb., 1942.

Pressures within the rumen of a cow were studied by means of a specially devised plug for a ruminal fistula. Belching occurs when the pressure is raised in the rumen by means of an active contraction of the ruminal musculature. Increased pressure, in itself, does not force gas from the rumen through the esophagus, as is illustrated by the artificial introduction of gas into the rumen. The amount of ruminal gas formed, both in a cow with a ruminal fistula and in a normal cow, was determined on rations of alfalfa hay alone, on green alfalfa alone, and on hay and grain. The amount of gas formed is more closely correlated with the time after eating than with the nature of the ration and is directly related to the amount of feed eaten. The composition of rumen gas was the same irrespective of the type of feeds in these experiments.

The theory is prosed that the expulsion of gas from the rumen by belching is a reflex mechanism dependent upon an adequate amount of fibrous material of a prickly nature. Succulent legumes and concentrates contain a minimum of fiber and are therefore particularly conducive to bloat. Preventive measures consist in the introduction of sufficient fiber in the ration of initiate belching. J.G.A.

689. Studies with Sulfabenamide. I. Blood Levels and Elimination in the Urine of Uninfected Rabbits. LORENZ HANSEN AND WILLIAM A. KREIDLER, Jefferson Med. Col., Philadelphia. Jour. Infect. Dis., *70*, No. 3: 208–214. May–June, 1942.

Methods are described for determining sulfabenamide in blood and urine. Tests following oral administration of the drug to uninfected rabbits revealed slow and incomplete absorption from the stomach. Absorption was more rapid following intraperitoneal administration. The rate of absorption and, with oral administration, the completeness of absorption, varied widely between animals; but for an individual rabbit were fairly constant during extended or repeated periods of administration. No severe

toxicity was found with a daily intake of 0.5 g. per kg. of body weight over a period of 1 week or with an intake of 0.25 g. over a period of 2 weeks.
J.F.C.

690. Studies with Sulfabenamide. II. Therapeutic value, Blood Levels, and Elimination in the Urine of Rabbits Infected with Beta Hemolytic Streptococci. Lorenz Hansen and William A. Kreidler. Jefferson Med. Col., Philadelphia. Jour. Infect. Dis., *70,* No. 3: 215–220. May–June, 1942.

Sulfabenamide was administered orally to 69 rabbits infected with beta hemolytic streptococci. A group of 40 infected animals served as controls. Of the treated group 60.9% recovered, but only 26.5% of the control group recovered. Heavy initial dosage with a gradual decrease was more effective than regular administration of intermediate amounts. Comparison tests showed that sulfabenamide had about the same effectiveness against beta hemolytic streptococcal infections as did sulfanilamide. The concentration of sulfabenamide in the blood and the rate of its excretion in the urine appeared to have no relation to survival time in the treated animals.
J.F.C.

FEEDS AND FEEDING

691. The Content of Metabolizable Energy in Feeds. (Fodrets innehåll av omsättbar energi.) Joel Axelsson. Kungl. Lantbruksakademiens Tidskrift, *80,* No. 5: 353–364 (English summary, 363). 1941.

The metabolizable energy in kcal. per g. digestible organic matter and the adjusted factors for the calculation of the starch value are as follows: (a) protein: coarse fodder 4.30, 1.14; concentrates 4.50, 1.20; (b) ether extracts: coarse fodder 7.80, 2.07; grains 8.30, 2.21; seeds of oil plants 8.80, 2.34; animal fodder 9.30, 2.47; and (c) carbohydrates: polysaccharides 3.76, 1.00; trisaccharides 3.62, 0.96; disaccharides 3.56, 0.95; monosaccharides 3.38, 0.90; nitrogen-free extract (average) 3.70, 0.98; crude fiber 2.90, 0.77, respectively. These values can be used for a satisfactory calculation of the contents of metabolizable energy for ordinary feeds, when the chemical composition and digestibility are known. The amount of metabolizable energy must be determined by respiration experiments, however, for such artificial products as "Strohstoff," "Dämpfstroh," cellulose and a few others. Since the amount of metabolizable energy is a straightline function of the contents of protein and crude fiber in the dry matter of the ration, the difference method gives reliable results, even for separate feeding stuffs added to the basal ration. The nutritive value of feeding stuffs for ruminants can, to advantage, be based on the contents of metabolizable energy. R.E.L.B.

692. Feeding Values of Silages and Hays. O. M. CAMBURN, H. B. ELLENBERGER, AND C. H. JONES. Vt. Agr. Expt. Sta. Bul. 482. March, 1942.

Results are set forth of sundry trials designed to indicate the comparative feeding values of corn and grass silages and of artificially dried and sun cured hays.

In 1935 pasture grass from a newly seeded pasture, cut in the early hay stage during a rainy spell and ensiled with three per cent molasses diluted with water was fed in comparison with corn silage to eight dairy cows. Its water content was high and its odor disagreeable. The average production of 4% milk equivalent per pound of total digestible nutrients when the grass and when the corn silages were fed were, respectively, 1.81 and 1.87 pounds, a non-significant difference. Live weight grains were much the same in each case.

In 1936–37 timothy grass in the full bloom stage, sun-cured; artificially dried; and ensiled with added molasses; were fed in comparison with corn silage to four groups of unbred heifers in two 120-day trials during two years. The average results, respectively, were 7.4, 6.8, 7.2, 6.0 pounds of total digestible nutrients consumed per pound live weight gain.

In 1939 timothy grass, grown during a rainy season, was stored as phosphoric-grass silage and as field-cured hay, the latter becoming somewhat musty. When fed to two groups of dairy heifers, the silage proved superior to the hay, the total digestible nutrient required per pound live weight gain being, respectively, 7.0 and 8.1 pounds. J.G.A.

FOOD VALUE OF DAIRY PRODUCTS

693. What Consumers Ought to be Told About Nutrition by Milk. HENRY T. SCOTT, Wis. Alumni Res. Foundation, Madison. Dairy World, *21*, No. 3: 14. Aug., 1942.

A very timely discussion of the place of milk and dairy products in maintaining nutritional efficiency in the armed forces and civilian populations. It is stated that milk protein is complete and of high nutritive value; milk sugar is possessed of nutritive qualities not found in other sugars; milk fats have been found superior in growth-promoting factors to most other fats; milk minerals, particularly calcium and phosphorus, are abundant and calcium is a limiting factor in many average diets; the vitamins A, thiamin, riboflavin and nicotinic acid are present in good quantity and act to supplement the lacks in other foods; vitamin D when added to milk is more effective than when incorporated in other foods; and the main item in a day's diet should be milk since it fortifies and balances the diet at so many points. F.J.D.

694. Milk in National Health. An interview with PROF. E. B. HART, Chairman of Section on Biochem., Univ. Wis. Wis. Med. Jour., *41,* No. 5: 402–406, 432. 1942. Indus. Med. *11,* No. 6: 290–292. 1942.

R.E.L.B.

695. Intestinal Implantation with *Lactobacillus acidophilus* by the Use of Bacillus Acidophilous Milk. J. D. WILLIS, Med. Arts Bldg., Roanoke, Va. Va. Med. Monthly, *68,* No. 6: 336–338. 1941.

A lecture. The addition of 4.5 g. of a superconcentrate of the *"Bacillus acidophilous"* (Farr Lab., Kalamazoo, Mich.) to 1 quart of whole milk, followed by incubation at 100° F. for 5–6 hours, produced a palatable, thick and comparatively sweet acidophilous milk with an acidophilous count of 800 million to 1 billion bacilli per cc. From 10 to 14 days are required, when 1 quart of acidophilous milk is consumed daily, to bring about a 50–60% intestinal implantation and 4 to 6 weeks to effect a 90% implantation. The implantation can be hastened by giving small doses of milk of magnesia daily as a mild laxative, also by adding lactose or dextrose to the diet. If a continuous implantation is desired, the feeding must be continued regularly; if discontinued, the intestinal flora will gradually revert to its former state. The use of acidophilous milk in various conditions is discussed briefly. R.E.L.B.

696. The Ascorbic Acid Content of Ewes' Blood, Colostrum, and Milk, and the Effect of Ascorbic Acid Injections. G. H. SATTERFIELD, E. A. BAILEY, JR., J. E. FOSTER, AND E. H. HOSTETLER, N. C. Agr. Expt. Sta., Raleigh. Jour. Nutr., *24,* No. 2: 121-219. Aug., 1942.

Twenty Hampshire ewes were used to study the ascorbic acid content of blood plasma, colostrum milk and normal milk, as well as the effect of intramuscular injection of ascorbic acid upon the ascorbic acid in blood plasma and milk. The ascorbic acid content of ewes' blood plasma was 0.43–0.83 mg. per 100 ml. The colostrum contained 2.01 to 9.94 mg. while normal milk contained 0.80 mg. ascorbic acid per 100 ml.

Intramuscular injection of 2 to 5 gm. of ascorbic acid resulted in a rise in the ascorbic acid level in the blood and milk. There was a wide variation in the magnitude of the rise in milk ascorbic acid in different animals.

C.F.H.

697. Where is Deficiency in Milk Drinking? L. D. WELD, McCann-Erickson, Inc. Milk Plant Monthly *31,* No. 1: 35. Jan., 1942.

A survey consisting of conversations with thousands of consumers, mostly housewives, showed a possibility of increasing milk consumption a total of 108% and 73.5% through milk drinking. Deficiency in milk drinking was manifest in the adult classes, 44.4% of the men and 49.4% of the women drinking no milk at all. Furthermore, the percentage of adult population is increasing yearly due to (1) decrease in birth rate, (2) longer life expectancy and (3) large immigrations in early decades. In 1900, 55.7% of the total population was adult; today 65.5% is adult. The reasons advanced by the adults for not using milk were (1) they disliked milk and (2) they could not afford it. Suggestions for milk promotional policy were: (1) stress importance of adequate daily amounts of milk, (2) direct the campaign largely to adults, (3) direct the campaign to both men and women, (4) feature milk drinking by showing how its nutritional value is greater than that of other beverages, (5) play up use of milk between meals and at bed-time, (6) appeal to the taste factor, (7) play up economy of milk, (8) tie up general nutrition story with the government program, and (9) use strong emotional appeals to urge action. G.M.T.

HERD MANAGEMENT

698. The Family Milk Cow on Georgia Farms. F. W. FITCH. Ga. Agr. Ext. Serv. Bull. 471. May, 1942.

A popular bulletin on the feeding and care of the family cow. Special features are schedules showing the feed needed by one cow for a year and the crops that may be sown under Georgia conditions to provide sufficient roughage and grazing for her. J.G.A.

699. The Cleaning and Bactericidal Treatment of Milking Machines. U. D. FRANKLIN, State Dept. Pub. Health. Jour. Med. Assoc. State Alabama, *11,* No. 7: 256–258. 1942.

A practical and effective method for the cleaning and bactericidal treatment of milking machines is described. R.E.L.B.

ICE CREAM

700. Sweetening Agents in the Manufacture of Ice Cream. KENNETH M. RENNER, Texas Technol. Col., Lubbock, Texas. South. Dairy Prod. Jour., *32,* No. 1: 32. July, 1942.

The results of studies on several sweetening agents for ice cream are briefly reviewed. The following conclusions are drawn by the author:

"From 25 to 30% of the sucrose (cane or beet sugar) can be replaced by corn sweetening agents without encountering any material difficulties in the rate of freezing, rate of whipping, or the rate of melting in the finished ice cream.

"When difficulties are encountered with 25 to 30% replacement with respect to rate of freezing, rate of whipping, and rate of melting, they can usually be overcome by using a colder freezing temperature, whipping the ice cream at a softer consistency than usual, and by maintaining a colder temperature in the ice cream hardening and dispensing cabinets.

"In high fat mixes dextrose used in place of a part of the cane or beet sugar will usually give a better flavor to the ice cream than is secured by cane sugar alone. In low fat mixes the flavor of the corn sweeteners is more readily detected.

"Dipping losses when corn sweeteners are used may be minimized by using a lower temperature in the ice cream cabinet. When the dipping temperature is lowered approximately one degree Fahrenheit for each one per cent dextrose added, no difficulty will be encountered.

"Other sweetening agents that may be used to replace a portion of the cane sugar in the ice cream mix are Invert Sugar, Honey, Ribbon Cane Syrup, and Sorghum Syrup.

"The following table represents data on sweetening agents used as compared to cane or beet sugar, the amounts to use and the percentage replacement the amounts to use and the percentage suggested."

Sweetener	Relative sweetness	Per cent moisture	Pounds required to secure same sweetening as 1 lb. of cane or beet sugar	Percentage replacement suggested
Cane or beet sugar	100	0.2	1.0	100
Corn sugar (dextrose)	83	7.2	1.1	25–30
Invert corn syrup (sweetose)	67	17.0	1.5	25–30
Corn syrup solids (Fro Dex)	49	3.5	2.0	25–30
Invert sugar sol.	140		1.0	25
Honey	70–75	17.5	1.28	25–50
Ribbon cane syrup	50		2.0	25
Sorghum syrup	50		2.0	25
Glucose	80		1.25	25

F.W.B.

701. **Cleaning Compounds for the Ice-Cream Industry.** D. V. JOSEPHSON, Pa. State Col., State Col., Pa. Ice Cream Field, *10*, No. 2: 8. 1942.

It is claimed that a good cleaning compound should have the ability to (1) soften water, (2) wet surfaces to be cleaned, (3) suspend dirt and other material in the washing solution, (4) emulsify fats, (5) rinse surfaces clean, and for certain purposes destroy bacteria.

Under the heading of basic alkalies the author describes sodium bicarbonate, sodium carbonate, trisodium phosphate, sodium hydroxide, and sodium metasilicate. It is stated that most commercially used water softeners are complex phosphates and tetrasodium pyrophosphate. Wetting agents are surface active materials which often exhibit great emulsifying powers. Sulphonated alcohols, ethers, hydrocarbons, and pine oil are classified in this group, and it is stated that their incorporation to the extent of 2–5% of the cleaning compound markedly improves the desirability of such preparations.

Various miscellaneous materials are added in the compounding of washing preparations. Thus indicators are added to give color; certain ammonium salts are frequently incorporated into mixtures intended for glass or dish washing; rosin, borax, and soaps are used in compounds for cleaning greasy floors; abrasive material is included for certain types of cleaning; acetic, tartaric and citric acids are widely used in removing milk stone compounds. Mention is also made of the use of acid detergents for can washing. The careful selection of washing preparations depending upon the nature of the cleaning problem and their efficient use is emphasized.

W.C.C.

MILK

702. A Study of Sonized Soft Curd Milk Prepared from Certified Milk. Harry S. Bikoff, Samuel S. Brown, Edward W. Zukauckas and Joseph C. Regan, Chairman. N. Y. State Med. Jour., *41*, No. 20: 2052–2057. 1941.

This report, made for the Milk Commission of the Medical Society of the County of Kings by a special committee (see above), is almost the same as that submitted to the Department of Health, New York City, on Feb. 1, 1940, except for a few minor changes. Sonic certified milk is an easily digested milk. The condition of comfort of the babies studied and the symptoms of crying, regurgitation, vomiting, abdominal distention, colic and hiccups so often associated with cow's milk, especially during the first few months of life, generally showed a rapid and marked improvement after sonized certified milk was started. R.E.L.B.

703. Homogenized Milk. A New Development in the Adaptation of Cow's Milk for Infant Feeding. Irving J. Wolman, Children's Hospital of Philadelphia, Philadelphia, Pa. Pa. Med. Jour., *44*, No. 6: 735–738. 1941.

An address. R.E.L.B.

704. Newer Developments in Quality Milk Production. John G. Hardenbergh, Walker-Gordon Lab. Co., Inc., Plainsboro, N. J. Jour. Med. Soc. N. J., *38*, No. 1: 20–23. 1941.

An address. R.E.L.B.

705. Bacteriological Aspects of Farm Milk Cooling. T. G. ANDERSON, Pa. State Col. Milk Plant Monthly, *31*, No. 8: 26. Aug., 1942.

In addition to a brief review of some of the more recent studies on farm cooling of milk the author reports results of bacteriological studies on the various levels of milk in 10-gallon cans under ordinary tank cooling and under adequate electrical cooling. Bacteriologically high-quality milk was often reduced to inferior quality in 12 hours by inadequate cooling. 90% of the organisms in a can of cooled milk were found to be in the top or cream layer probably as a result of (1) the straining out of the contained bacteria by the rising fat globules, (2) favorable oxygen relationships for bacterial growth, (3) a sufficient food supply for bacterial growth, and (4) a slow rate of cooling at the surface permitting sufficiently high growth temperature. Farm milk cooling by the tank method with water above 50° F. and without agitation did not afford adequate protection against bacterial growth whereas electrical farm milk cooling of evening's milk was adequate from the bacteriological standpoint. Cooling milk entirely and quickly to a temperature below 50° F., preferably 40° F. is advised. Temperature increase during two-hour transportation on an open truck ranged from 12 to 21° F. depending upon the air temperature and the proximity of cold cans to warm cans of morning milk. G.M.T.

706. The Behaviour of Resazurin in Milk. C. K. JOHNS, Dom. Dept. Agr., Ottawa, Canada. Cada. Jour. Res., *20*, No. 6: 336. June, 1942.

The view has been expressed (see JOURNAL OF DAIRY SCIENCE, Abs. No. 936, p. A374, 1941) that resazurin is not a satisfactory substitute for methylene blue in assessing the hygienic quality of milk.

Additional evidence is presented that resazurin is equally as sensitive as methylene blue to the metabolic activities of bacteria in milk. It is decidedly more sensitive to the presence of non-bacterial factors in abnormal milks (mastitis, late lactation, etc.) and therefore furnishes a more comprehensive index of the true quality of the milk.

No support was found for the view that resazurin exerts such a strong poising action in milk as to interfere with the interpretation of results, nor were milks found to vary significantly in their poising properties. Time-potential curves for milks containing methylene blue, resazurin and various reducing substances are presented as well as those of milks from abnormal udders. O.R.I.

707. Paying Producers for Fat and Solids-not-Fat in Milk. RUDOLPH K. FROKER AND CLIFFORD M. HARDIN. Wis. Agr. Expt. Sta. Res. Bul. 143. Feb., 1942.

The purchase of milk with consideration to weight and fat content alone is not satisfactory, especially under present conditions of wide fluctuations in government purchases. This bulletin is concerned with an equitable method of paying for both the fat and solids-not-fat in milk used for any purpose.

Consideration was given to the fact (1) that for each 0.1% variation in fat there is a corresponding change of 0.04% in the solids-not-fat, (2) the market value of each product into which the milk is made must be taken into account, and (3) certain costs of handling and manufacture must be deducted. In final form the milk is paid for on the basis of a definite flat rate per hundredweight of milk with a proper fat differential, these prices depending upon the market value of the products for which the milk is used.

Illustrations are given for milk prices for creameries, cheese factories, condenseries, and fluid milk markets. The primary value of the methods lies in the consideration given to the composition of the milk, the economic value of the products manufactured, and the interlocking of these values into one price per hundredweight with a fat differential.

The original bulletin must be studied for details of the calculations.

A.C.D.

708. Efficiency of Milk Marketing in Connecticut. (2) Transportation of Milk. D. O. HAMMERBERG AND W. G. SULLIVAN. Storrs Agr. Expt. Sta. Bul. *238*. 1942.

This study led to three major conclusions: (1) Milk transportation is inefficiently organized. (2) The charges made for hauling milk are not in proportion to the costs of performing the service. (3) A monopolistic situation frequently exists as far as the sale of milk is concerned which often leads to charging excessive hauling charges.

The author recommends consideration of public regulation of the hauling of milk so as to eliminate excessive charges and to bring about greater efficiencies by consolidating routes and eliminating the inefficient ones. The concentration of the assembly function in the hands of a private company was suggested as the most desirable form of control. In order to achieve the most efficient organization it is recognized that there must be full participation from all producers concerned. P.H.T.

709. New Method of Cleaning Milk Cans. V. SCHWARZHOPF, Lathrop-Paulson Co. Milk Plant Monthly, *31*, No. 1: 50. Jan., 1942.

A good cleaning agent should be (1) effective in removing milk fat, milk solids and other foreign material quickly, (2) be effective in emulsifying the loosened particles for quick removal, (3) prevent the formation of sludge, (4) rinse freely and completely, (5) prevent scale deposit on can and machine, (6) not have any injurious effect upon the tinned surface of the can or the metal in the machine, (7) be of low cost so wash and rinse water can be continually changed to prevent accumulation of milk fat and solids in wash and rinse water, (8) be easily prepared and utilized, (9) deliver finished can either with neutral or slightly acid surfaces, (10) be non-toxic, and (11) accomplish all the above at temperatures at or above the boiling point as such temperatures are desirable for effective sterilization.

The new method of washing milk cans, called the conservation method because it conserves steam, water and detergents, embodies the use of an acid detergent. Sufficient acid detergent is added to bring the wash water slightly under pH 7, which is maintained throughout the washing process. Results obtained indicate that (1) cleaner and more nearly sterile cans are obtained, (2) the numbers of thermoduric, thermophilic, lipolytic and proteolytic bacteria are greatly reduced, (3) the reduction in cost of washing, sterilizing and drying cans is enormous, (4) the receiving room is quieter and more comfortable because of reduction in amount of steam used and elimination of the steam jet for sterilization, (5) and that exhaust fans are not needed in the vent pipe of the can washer. Assumptions are made that sterile cans may soon replace relatively sterile cans, that costs will be further reduced and that can life will be prolonged. G.M.T.

710. Relation of Chocolate Milk to Total Fluid Consumption.

M. A. SCHAARS, Dept. Agr. Econ., AND G. HADARY, Univ. Wisc., Madison. Milk Plant Monthly, *31*, No. 3: 28. March, 1942.

A survey was made to determine what differences exist in total milk consumption among families buying chocolate milk and those who do not. The study was made with a test city of 60,000 population. The results showed that on a fluid milk basis the per capita milk consumption among chocolate milk buyers was 21% greater than that of non-purchasers of chocolate milk. A study of per capita consumption of chocolate milk among the different income groups was made and findings showed that there were 29%, 33% and 14% increases among the high, medium, and low income groups, respectively.

There was a tendency for those using chocolate milk to be more uniform in their milk drinking habits than the non-users of chocolate milk. These findings have been substantiated by work in other cities. G.M.T.

711. Ways of Conserving Tires and Reducing Other Expenses in the Distribution of Milk.

R. ANDERSON AND L. SPENCER, Dept. Agr. Econ. and Farm Mangt., N. Y. State Agr. Col., Cornell Univ., Ithaca, N. Y. Milk Plant Monthly, *31*, No. 3: 24. March, 1942.

The adoption of alternate-day delivery offers the most promising change in milk delivery systems from the viewpoint of tire conservation and cost saving. Introduction of a schedule of discounts for volume delivery to consumers makes it possible for any distributor to adopt the system whether competitors follow out the plan or not. The volume discount system or use of larger-than-quart-size containers results in considerable saving of delivery time though not in truck mileage.

"Smaller but considerable savings can be made in other ways: (1) By discounting or exchanging customers that are expensive to serve, such as customers at a distance from main route, single or scattered stops in apartment houses, and split stops as stores, restaurants, etc.; (2) By drastically curtailing call-backs and special deliveries; and (3) By changing from early-morning to daylight delivery." G.M.T.

712. Production and Prices of Milk in the Springfield-Holyoke-Chicopee Milkshed in 1935. A. A. BROWN AND M. BOOTH. Mass. Agr. Expt. Sta. Bul. 389. Feb., 1942.

This is the third in a series of statistical studies pertaining to the milk supply of the area indicated (see Mass. Bul. 363 and 364). The most important conclusion reached has to do with price-fixing policies. The studies indicate that for such secondary markets the policy of pricing milk F.O.B. the farm has much in its favor, as contrasted with the present practice of fixing prices F.O.B. the market. It is claimed that price fixing F.O.B. the farm would result in lower transportation costs and greater market stability, in addition to lifting from the producer the burden of ofttimes excessive costs resulting from the present system.

It is also pointed out that relatively low per capita milk consumption is due largely to economic causes and that under our present distribution system remunerative prices to producers and simultaneously attractive prices to consumers are incompatible. If the consumption of milk is ever to reach even the minimum suggested by nutritionists as essential in the daily diet, the costs of servicing must be reduced either by public subsidy or by eliminating some of the services now performed and performance of others by the consumer. "It seems unlikely that anything but outright public support of distribution will encourage the increase in consumption that nutritionists say is desirable." J.G.A.

713. Efficiency of Milk Marketing in Connecticut. D. O. HAMMERBERG, L. W. PARKER, AND R. G. BRESSLER, JR., Univ. Conn. Univ. Conn. Bul. 237. 1942.

Since most dealers buy milk on the use classification basis, there are a great many different prices in each metropolitan district. This condition is reflected in the irrational and inefficient location of producers and of milk sheds.

The results of the study show that it is possible to allocate producing areas to milk markets in a manner that will minimize the costs of moving milk from farms to markets.

Further economics would result from the addition of country separating plants in several sections of the state.

With optimum organization of trucking, the revision of areas would decrease the total distance traveled nearly 16% or approximately 550,000 miles per year. P.H.T.

714. Reducing Truck Mileage in Retail Milk Delivery. J. A. HITCHCOCK, Vt. Agr. Expt. Sta. Vt. Agr. Expt. Sta. Bul. 491. 1942.

It is estimated that 80% of the present truck mileage in house door delivery is unnecessary. An elimination of all duplication could be accomplished only by placing the delivery in the hands of one agency.

Nearly all stores selling milk in the area studied (Burlington and Winooski) have sufficient refrigerator space to store two days' supply of milk and cream. Practically 96% of the homes in Burlington and Winooski have either electrical or ice refrigeration.

These studies were made for the purpose of considering the feasibility of every other day delivery of milk. P.H.T.

715. Bottled Milk Deliveries. H. B. ELLENBERGER, Univ. Vt. Univ. Vt. Bul. 486. 1942.

A study of the delivery systems used by milk dealers of Burlington, Vermont, has shown that 41 routes covered the same territory in many cases. The delivery equipment of 21 to 37 competing companies was found to operate on some of the same streets. Data regarding the amount of milk and cream delivered, the distance traveled, number of stops, and time required was secured on the different routes studied. P.H.T.

PHYSIOLOGY

716. Lactational Performance and Body Weight. SAMUEL BRODY, Dept. Dairy Husb., Univ. Mo. Science, *95*, No. 2471: 485–486. 1942.

The relation of milk-energy production to body weight in mature cattle, dairy goats and white rats is shown graphically. The data are generalized by the equation, $Y = aW^b$, where Y represents milk-energy production and W body weight. The precise numerical value of the slope b varies with the relative "dairy merit" of the animals. The significant fact is that milk-energy production tends to vary with W^b and the value of b is of the order of 0.7 ± 0.1. Within a given gross energetic-efficiency class of milk production (ratio of milk-energy produced to digestible feed-energy consumed), the larger the animal the less the labor cost per unit milk-energy produced and, if other conditions are equal, the greater the profit per unit milk produced, and still greater per animal and per herd. However, it is not known how total maintenance cost varies with increasing body weight. The gross energetic efficiencies of milk production in the small animals were higher than in the large: cows 31%, goats 35% and rats 44%. These differences may be fortuitous, but they may also indicate that total maintenance-energy cost rises more steeply with increasing body weight than milk-energy production, due either to selection factors or to physicochemical interrelations having a similar effect. R.E.L.B.

717. **The Effect of Nicotine on Lactation in White Mice.** J. ROBERT WILLSON, Dept. Obstet. and Gynec., Univ. Mich. Med. School. Amer. Jour. Obstet. Gynec., *43*, No. 5: 839–844. 1942.

The effect of the daily oral administration of 0.5, 1.0, or 2 0 mg. nicotine on lactation, as shown by the weight gains in the offspring, was studied in white mice through 3 consecutive litters. The litter size showed a slight but consistent reduction with increasing doses of the drug. However, the controls and the treated animals exhibited no remarkable differences in mortality rate, birth weight or weight gain during the period of lactation All these factors varied markedly within each individual group It is concluded that lactation in white mice is not impaired by the ingestion of nicotine. R.E.L.B.

718. **The Inhibition of Lactation During the Puerperium by Methyl Testosterone.** PAUL M LASS, Dept. Obst. and Gynec, Columbia Univ. and the Vanderbilt Clinic. Amer. Jour. Obstet. Gynec., *43*, No. 1: 86–93. 1942.

Methyl testosterone, in adequate dosage and given at the proper time, is of definite value in the postpartum inhibition of lactation. The optimal oral dosage is about 250–300 mg. in divided doses over a period of 36 hours, starting about 36 hours after delivery. It may also be administered after the actual onset of secretion with beneficial results. There were no associated symptoms of intolerance. R.E L.B.

719. **Ovulation and Its Relation to Estrus in Cows.** A. NALBANDOV AND L. E. CASIDA, Univ. Wis, Madison. Jour. Anim. Sci., *1*, No. 3. 189–198. Aug., 1942.

A total of 72 estrual periods on 22 grade dairy cows were studied. There was a marked tendency for ovulation to occur after the end of estrus, usually approximately 14 hours. The mean time of day for estrus to end was between 8 and 9 P.M., while the mean time of ovulation was approximately 11 A.M. The later the hour that heat ended the shorter the interval from the end of heat to ovulation. The later the hour of ovulation the longer the interval from the end of heat to ovulation. The later the hour that heat ended the later was the hour of ovulation. C.F.H.

720. Correction of Heat Production for Changes in Live Weight of Cattle in Balance Experiments. R. W. SWIFT, Pa. Agr. Expt. Sta., State College. Jour. Anim. Sci., *1*, No. 2: 145–148. May, 1942.

A method of correction of heat production of cattle on account of changes in live weight in the course of energy metabolism experiments is presented. This correction is based on the balances of carbon and nitrogen coincident with the changes in live weight. It is based upon the results of the balances of nitrogen and carbon rather than live weight changes as indicated by scales.

Data are presented which illustrate that animals may be losing weight according to the scales but actually gaining in weight of body tissue as shown by carbon and nitrogen balances. The variation in the fill of cattle results in an error when body weights are measured by scales. C.F.H.

721. Milk Yields and Milking Rates of the Individual Quarters of the Dairy Cow Udder. C. A. MATHEWS, W. W. SWETT, AND R. R. GRAVES. U. S. Dept. Agr. Tech. Bul. 827. Oct., 1941.

Ninety-four cows milked twice a day with a combine milker were used in this study. Average yields from each quarter in terms of total yield were: left rear, 29.2%; right rear, 29.0%; right front, 21.6%; left front, 20.2%. Milk yields of opposite quarters were frequently not alike. The yield data indicate that the differences in relative milk yields from front and rear quarters may be more pronounced between families than between breeds.

Changes in milking rates brought about by different conditions show that the front and rear quarters were affected in very much the same manner. Certain characteristics of the milking process are common to all quarters of the udder in machine-milked cows. Not only do milking rates vary during the course of a single milking, but they also vary from one milking to another if there are differences in the total milk yields. Higher milk yields result in higher rates in pounds per minute, and whether from separate quarters or for the entire udder also require a little longer milking time, but the percentage increase in time required is much less than the percentage increase in milk yield. J.G.A.

MISCELLANEOUS

722. Introducing "Foamglas"—A New Insulating Material. ANONYMOUS. Ice and Refrig., *103*, No. 1: 9. July, 1942.

Foamglas is a product introduced by the Building Materials Division of the Armstrong Cork Company. It is a non-priority product, consisting of glass in block form which weighs only 1/15 as much as ordinary glass. It is said to be a perfect vapor barrier. It is fire and waterproof. It will not rot, mold or decay. It is vermin proof and odorless. It is manufactured by firing ordinary glass mixed with pure carbon. As the carbon is converted into a gas a cellulated product is obtained. L.C.T.

723. Frozen Fruit and Vegetable Research Indicates Desirable New Products for Lockers. H. H. PLAGGE AND BELLE LOWE, Iowa Agr. Expt. Sta., Ames, Iowa. Ice and Refrig., *102,* No. 6: 357. June, 1942.

To the dairyman this is an informative article if, in connection with his dairy business, he also operates a food locker plant. It discusses the various varieties of vegetables and fruit, their growing, packing and storage characteristics, together with a description of the final product that may be expected after locker storage. Convenient tables giving these various characteristics are included. L.C.T.

724. Insulation of Cold Pipes. ANONYMOUS. Ice and Refrig., *103,* No. 2: 105. Aug., 1942.

The article in question deals with the use of Mineral Wool Felt and has been prepared by the Industrial Mineral Wool Institute. The subject matter of the article is broken down into eight headings: (1) Recommended thickness, (2) preparation of surface, (3) application of mineral wool felt, (4) fittings, (5) finish on straight piping, (6) finish on valves and fittings, (7) finish on valves and fittings (alternate), (8) plumbing.

Since the article is in outline form, it is inadvisable to discuss each of the above in this abstract. Item number 1, however, may be quite valuable for general reference and is therefore reproduced.

Operating Temperature	Total Built up Isulation Thickness
45° F. to 15° F.	2 inches
15° F. to − 5° F.	3 inches
− 5° F. to −20° F.	4 "
−20° F. to −40° F.	5 "
−40° F. to −60° F.	6 "

L.C.T.

725. Conserving Equipment. E. C. DAMROW, Damrow Bros. Co., Fond du Lac, Wis. Dairy World, *21,* No. 3: 20. Aug., 1942.

The author discusses the conservation of cheese plant equipment in the light of the extreme shortages of steel, copper, tin and other metals. The necessity for replacing 800 cheese vats every year could be cut down fully one-third and other equipment in proportion if conscientious efforts were made. It is suggested that vats be taken apart and cleaned at least once a year and the metal shell repaired and painted on the jacket side. Cheese hoops should be cleaned and oiled before putting away for the season. Separators should run smoothly. Bearings should be frequently checked. Discs should be tight in the bowl and not allowed to rust. Can washers last only as long as they are kept in good mechanical operating condition and unless they deliver a dry can, the can will rust. It is suggested that the slack season be utilized in servicing, repairing and conditioning cheese plants and equipment to eliminate any possibility of breakdown or failure next spring when quick repairs and replacement may be impossible. F.J.D.

726. New Material for Milk Plant Floors. L. D. Dunn, Creamery Package Mfg. Co. Milk Plant Monthly, *31*, No. 1: 41. Jan., 1942.

A satisfactory flooring for creameries and milk plants should be (1) economical, (2) hard, long wearing, (3) non-porous and, (4) non-slippery. Types of floors that are in common existence now are those made of concrete and tile. A relatively new floor which possesses the properties of a satisfactory floor is an Emery Aggregate concrete topping. This floor surface resists lactic acid attacks, is non-porous, tough, and extremely hard. Other properties of this floor is its great tensile strength and its ability to absorb but little moisture.

This covering may be satisfactorily used on old base slabs as well as new ones, and for patching. A covering of ¾ inch is satisfactory for most purposes, while a thickness of 1 inch is used on extra heavy duty floors such as loading platforms and receiving rooms. Various color combinations may be used for floors in lobbies, reception rooms and offices. G.M.T.

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ABSTRACTS OF LITERATURE

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ABSTRACTS OF LITERATURE

BOOK REVIEWS

727. Pumps, Types, Selection, Installation, Operation, and Maintenance. Frank A. Kristal, Mech. Eng., Weinman Pump Mfr. Co., and F. A. Annett, Assoc. Ed., "Power." McGraw-Hill Book Co., Inc., New York. 1940.

These authors have attempted to cover the subject of pumps from the standpoint of practicality, that is, operational application, rather than the most common treatment encountered, that of design problems. Therefore, after a description of the many industrial types of pumps, including operating principles and construction materials, comprising the first ten chapters, the authors take up in the succeeding eight chapters the subjects: Methods of Priming Pumps; Pump Operations; Drives for Pumps; Pump Selection, Installation, and Operation; Centrifugal-pump Troubles and Remedies; New Pumps for Old. This book contains much information which should prove valuable to the dairy plant operator and to the dairy equipment sales engineer. Very well illustrated. L.M.D.

728. Industrial Chemistry of Colloidal and Amorphous Materials. Warren K. Lewis, Mass. Inst. Technol., Lombard Squires, DuPont de Nemours Co., and Geoffrey Broughton, Eastman Kodak Co. The Macmillan Co., N. Y. 1942.

This book prepared as a text on the industrial chemistry of colloidal and amorphous materials is of particular interest to those in the dairy industry engaged either in research along such lines or in the technical applications of research findings because of its clear-cut treatment of the basic physical chemistry phenomena involved and understanding of which is necessary in the interpretation of industrial problems arising in technical applications. Thus the first half of the text is devoted to such subjects as: structure of liquids, viscosity, surface tension, surface tension and orientation, adsorption, suspensoids, amorphous solids, emulsoids, the electrochemical behavior of colloids, gelation, emulsions and foams, and crystalline and amorphous states. The balance of the subject matter is devoted to industrial aspects largely outside of the dairy field. L.M.D.

BACTERIOLOGY

729. An Emergency Method for Estimating Bacterial Populations (A Preliminary Report). Ray Hasson, Griffin-Hasson Lab., Los Angeles, Calif. Jour. Milk Technol., *5*, No. 4: 243. July-Aug., 1942.

Ten milliliters of standard agar media (2% agar) is sterilized in two-ounce square-bottomed flint glass bottles fitted with molded bakelite caps. While agar is still melted, the bottles are laid on their sides until media has solidified. The agar is then flooded with the samples of milk to be examined, drained and then incubated. Sterile skimmilk is recommended for making dilutions when necessary.

The quantitative results obtained may be correlated with an actual plate count and a factor obtained which may be used for future determinations under like conditions.

No mention is made of the amount of milk used for flooding the agar slab nor the length of time it should be drained. L.H.B.

730. **Detection of Thermoduric and Thermophilic Bacteria.** A. MOLDAVAN, Guaranteed Pure Milk Co., Ltd., Montreal, Canada. Canad. Dairy and Ice Cream Jour., *21,* No. 4: 36. 1942.

In the simplified technique described in this paper the three usual operations, test-tube pasteurization, diluting or looping, and agar-tubing may be carried on simultaneously. When more specific information is desired, four sets of separate tests may be carried on simultaneously to differentiate true thermodurics from milk-protected thermoduric and thermophilic bacteria and at the same time establish the rate of survival of such types under laboratory conditions. These tests are raw milk count, laboratory-pasteurized milk count, laboratory-pasteurized 0.001% dilute milk count, and laboratory-pasteurized milk count incubation at 60° C. The calibration and use of a special platinum loop is described. Bacteria surviving pasteurization temperatures in a 0.001 ml. milk dilution in distilled water may be termed true thermoduric and often are similar in types to those found in poorly sterilized cans. O.F.G.

731. **Biennial Reviews of the Progress of Dairy Science. Section B: Bacteriology and Mycology Applied to Butter.** ANONYMOUS. Jour. Dairy Res., *12,* 350–390. 1941.

Recent pertinent literature is reviewed under the headings: (1) Milk control, technique (90 references); (2) Lactic acid and allied fermentations, (a) bacterial metabolism, (b) lactic acid bacteria, (c) starters, (d) cheese, (e) butter (228 references); (3) Pasteurization and other processes, (a) pasteurization, (b) other processes, (c) canned and dried milk (65 references); (4) Detergents and disinfectants (24 references). S.T.C.

BUTTER

732. **Reducing the Loss of Fat During Churning.** G. H. WILSTER, R. P. ROBICHAUX, R. E. STOUT, AND R. W. STEIN. Oregon State College Station Bul. 397. July, 1941.

This study was based upon observed butterfat losses in buttermilk in

Oregon creameries and the influences of specified recommended practices upon these losses. The percentage of fat in the buttermilk was determined by the Babcock-butyl-alcohol-sulphuric-acid method of McKay and Mitchell and the results agreed well with those secured by the Mojonnier method. The results were expressed as the per cent of the total churned fat which was lost in the buttermilk.

In the college creamery and well-operated commercial creameries from 1.0 to 1.25% of the fat in the cream was lost in the buttermilk. These losses are considered to be minimum values. When losses of fat were great they could be reduced by several simple practices. The cream should test 36 to 40% fat. Low test cream gives more buttermilk that tests higher in fat. The water rinse should not be added directly to the churn but should be separated and this cream used for churning. The pasteurized cream should be cooled to 40°–45° F. and held at least three hours before churning, preferably overnight. The churn should not be overloaded and the cream churned at a temperature to require at least 45 minutes to churn. Some plants were found that were losing a minimum of fat while one creamery saved $20 per day through more exhaustive churning by following these recommendations.

A.C.D.

733. **The Rheology of Butter. I. Methods of Measuring the Hardness of Butter.** R. M. Dolby, Dairy Res. Inst. (N.Z.). (D.S.I.R.) Palmerston North, New Zealand. Jour. Dairy Res., *12,* 329–336. 1941.

An apparatus is described in which the resistance of butter to cutting by a wire is measured. The results are expressed as the load in grams required to produce a given rate of cutting. The results were found to be in general agreement with, but much more reproducible than those secured using the Scott Blair apparatus in which hardness is measured by compression of a cylindrical sample. S.T.C.

734. **The Rheology of Butter. II. The Relation between the Rate of Shear and Shearing Stress. The Effect of Temperature and of Reworking on Hardness and on Structural Viscosity.** R. M. Dolby. Dairy Res. Inst. (N.Z.). (D.S.I.R.) Palmerston North, New Zealand. Jour. Dairy Res., *12,* 337–343. 1941.

It is shown that the rate of shear of butter when cut by a wire increases as a power of the load applied to the wire. The values determined for this power were high (index $n = 8$ to 15, average 11 at 12.5° C. (54.5° F.) and show a high structural viscosity in the butter. Although there was no indication that butter exhibits a true yield value the (rate of shear)/(load) curve is such that a practical yield value can be found. The practical yield

value (defined as the minimum load required to produce a measurable rate of flow) was used as a measure of the hardness of butter. The hardness of a number of samples of butter was determined over the range 6–18° C. (42.8°–64.4° F.). The hardness of the butters showed a steady fall with increase in temperature but the rate of decrease was not the same for all the samples so that their order of hardness changed as the temperature was increased. Reworking was shown to decrease the hardness to about one-fourth of the original value. There was a slow recovery on standing.
S.T.C.

735. **The Rheology of Butter. III. The Effect of Variation in Butter-making Conditions on the Hardness of the Butter.** R. M. DOLBY. Dairy Res. Inst. (N.A.). (D.S.I.R)Palmerston North, New Zealand. Jour. Dairy Res., *12,* 344–349. 1941.

Slowly cooled cream was found invariably to yield butter which was softer than that churned from rapidly cooled cream. The use of low temperature wash water tended to reduce the hardness of the butter but the effect was small. The other factors studied had no consistent effect on the hardness of the butter. These were the type of pasteurizer, temperature during holding and during churning and amount of working. S.T.C.

736. **Observations on Bacterial Discoloration of Butter.** A. H WHITE, Dairy Specialist, Sci. Serv., Ottawa, Canada, Canad. Dairy and Ice Cream Jour., *21,* No. 7: 19. 1942.

Discoloration of butter which contained from 1.5 to 2.0% salt has been found due to *Pseudomonas nigrifaciens,* although on laboratory media the organism does not produce pigment with 5.0% salt or more. The defect was also reported for the first time on the surfaces of butter boxes which had been held at temperatures of 33° to 40° F. Case histories and experimental trials indicated that the discoloration had developed because of extraneous moisture at the surface of butter. The organism has been isolated from floors, drains, and a butter printing table in plants which have experienced trouble. The organism is easily destroyed by pasteurization at 170° F. for one minute. O.F.G.

737. **What War Has Done to Fats and Oil Supplies.** G. W. MCBRIDE. Food Indus., *14,* No. 2: 57–59. 1942.

Warfare in the Pacific has reduced our imports of fats and oils. Primary fats and oils have not been increased in production domestically to meet our needs during the past 30 years.

In 1942, however, a 35% increase in lard production over the 1936–40 average is anticipated. Flax seed production in 1942 will approach record production; and Canadian and Argentine surpluses are available.

The production and trade in oils indirectly affect milk fat economics. Regardless of the balance maintained between animal and vegetable fats, specific fats will be subject to shortages although there is, by a price-fixing plan, a tendency to reduce shortages in the fat and oil supplies. J.C.M.

CHEESE

738. How to Use Waste Rind in Processed Cheese. SIMON BRICKNER, Consulting Chemist, Brooklyn, N. Y. Food Indus., *14,* No. 3: 47. 1942.

Natural Swiss cheese is covered with a rind a quarter of an inch thick. Natural Swiss cheese is used in making processed Swiss cheese and part of a blend of American cheese. Before cooking the rind has usually been thrown out but it has been found to contain 35% fat and 20% moisture. This is ideal for processing. In a special kind of hammer mill it can be ground to a fine powder and up to one third of a batch replaced by it without bad results. A higher overrun is possible because of its low moisture content.

This process used commercially for past one and a half years has been very gratifying. It may lower cost of production and increase the amount of processed cheese. J.C.M.

739. A Simplification of the Scott Blair—Coppen Test for the Pitching Consistency of Cheese Curd. G. W. SCOTT BLAIR AND F. M. SCOTT BLAIR. Natl. Inst. Res. in Dairying, Univ. Reading, Eng. Jour. Dairy Res., *12,* 322–328. 1941.

The simplification of the Scott-Blair Coppen test for the pitching consistency of cheese curd (reviewed in JOUR. DAIRY SCI., *24,* A26, 1941) consists in increasing the time for each stage in the process. The test previously carried out in 50 seconds, each process being carefully timed to the nearest second, can be as effectively done more slowly if the same proportionate times are used for each stage. The timing need not be highly accurate.

S.T.C.

740. Organisms Causing Rusty Spots in Cheddar Cheese. C. S. PEDERSON AND R. S. BREED, N. Y. State Agr. Expt. Sta., Geneva, N. Y. Canad. Dairy and Ice Cream Jour., *21,* No. 8: 19. 1942.

Rusty spot outbreaks in cheddar cheese have been reported from time to time and according to the results of the study reported in this article are caused by chromogenic varieties of *Lactobacillus plantarum* var. *rudensis* and *Lactobacillus brevis* var. *rudensis.* The first organism produces a trace of carbon dioxide in its production of lactic acid from glucose and other carbohydrates, whereas the second produces, in addition to lactic acid, carbon dioxide, alcohol, and volatile acids from dextrose and mannitol from levulose. Non-chromogenic strains of the two organisms are commonly present during the ripening of all cheddar and similar types of cheese.

O.F.G.

741. **Some Other Activities of the Dairy Products Board.** J. F. Singleton, Chairman, Dairy Products Board. Canad. Dairy and Ice Cream Jour., *21*, No. 4: 19. 1942.

The Board has been active in promoting the saving of domestic veals suitable for the manufacture of rennet, has purchased 100,000 veals from New Zealand, and has distributed these at cost to the two Canadian manufacturers of rennet. Provisions are being made to substitute pepsin for rennet in the manufacture of cheese if it becomes necessary even though pepsin does not make as satisfactory cheese as rennet. The Board also has been active in securing the release of black iron, tinned iron, and block tin for the construction of cheese vats and milk cans. There has been a shortage of properly seasoned cheese boxes.

Milk production during 1941 exceeded that of 1940 by about a billion pounds or about 6%. An increase of 500 million pounds for 1942 over 1941 seems desirable to take care of the increased domestic demands and to provide 125 million pounds of cheese for the United Kingdom. O.F.G.

CHEMISTRY

742. **Effect of Light on Riboflavin Solutions. Effect of Sunlight on Reduced and Unreduced Solutions.** C. M. O'Malley and C. W. Sievert. Amer. Dry Milk Inst., Inc., Chicago, Ill. Jour. Indus. and Engin. Chem., Indus. Ed., *34*, No. 9: 1117. Sept., 1942.

Two extracts of skim milk solids and one solution of synthetic riboflavin were prepared for 30 minute periods of exposure to sunlight. To a portion of each extract, sodium hydrosulfite was added to maintain the riboflavin in a reduced state during exposure. About 90% of the riboflavin in the unreduced solutions was destroyed by the exposure but only a small portion of the reduced riboflavin was lost. Reduction of riboflavin with an unstable reducing agent did not protect the solution when heat was applied.

B.H.W.

743. **Measuring the Concentration of Dissolved Oxygen in Dairy Products. A Voltammetric Method.** G. H. Hartman and O. F. Garrett, N. J. Agr. Expt. Sta., New Brunswick. Jour. Indus. and Engin. Chem., Analyt. Ed., *14*, No. 8: 641. Aug., 1942.

The method of measuring dissolved oxygen by the dropping mercury electrode has been applied to milk. The method determines the concentration of dissolved oxygen in the aqueous phase of a milk product and the quantity of dissolved oxygen is calculated after considering the solids content of the product. The slight variations in the solids content of normal milks did not introduce significant errors. A linear relationship was observed between the concentration of dissolved oxygen in milk and the magnitude of the galvanometer deflections. The concentration of oxygen in milk may be determined at a potential ranging from 0.8 to 1.2 volts. The method gave reliable results when used in the determination of dissolved oxygen in a number of commercial milk samples. B.H.W.

744. Oxidative Destruction of Vitamin D. J. C. Fritz, J. L. Halpin, J. H. Hooper, and E. H. Kramke. The Borden Co., Elgin, Ill. Jour. Indus. and Engin. Chem., Indus. Ed., *34*, No. 8: 979. Aug., 1942.

A substantial loss in potency may occur in the vitamin D added to dry carriers, such as dry whey, which are used in mixed feeds. Some feeds show marked loss of vitamin D during three-month storage at room temperature; others show no loss during six-month storage. All the sources of vitamin D tested were susceptible to destruction. There was little evidence of a lag or induction period in this destruction. Oxidation was shown to be the cause of vitamin D loss and any condition which promoted oxidation such as increased surface area or the presence of fat which became rancid during the storage period accelerated the destruction. Various methods of stabilization were tested. Protection resulted from elimination of air contact and storage at low temperatures. Different coating agents such as calcium stearate were found to effectively retard oxidation when they were used to form a protective coating around the feed particles. If the impregnated particles were completely covered, the nature of the insulation had little effect on the protection afforded. Antioxidants such as oat flour were helpful in retarding oxidation. B.H.W.

745. Extraction and Assay of Nicotinic Acid from Animal and Plant Tissues. Comparison of Methods. Veron H. Cheldelin and Robert R. Williams, 297 Summit Ave., Summit, N. J. Jour. Indus. and Engin. Chem., Analyt. Ed., *14*, No. 8: 671. Aug., 1942.

Various aspects of the problem of determining nicotinic acid in a number of natural substances have been studied. Seven enzymes were employed for digestion, takadiastase and papain appearing to liberate nicotinic acid completely from a variety of materials. Enzymatic digestion or acid or alkaline extraction gave the same values for nicotinic acid content of meats and milk while with cereals the acid and alkaline treatments gave higher values. B.H.W.

746. Growth Stimulants in the Microbiological Assay for Riboflavin and Pantothenic Acid. J. C. BAUERNFEIND, A. L. SOTIER, AND C. S. BORUFF, Hiram Walker and Sons, Inc., Peoria, Ill. Jour. Indus. and Engin. Chem., Analyt. Ed., *14*, No. 8: 666. Aug., 1942.

Stimulants for the growth of *L. casei* in the microbiological assay for riboflavin and pantothenic acid when suboptimum amounts of the vitamin are present in the assay medium have been shown to exist in some foodstuffs including dried skim milk. The presence of the stimulants has been found responsible for discrepancy in results in assay of riboflavin and pantothenic acid. Stimulatory action of aqueous extracted residues, photolyzed aqueous suspensions of foodstuffs, extracted residues of distillers' dried solubles, and a number of known compounds is cited. The stimulatory action may be avoided by use of clarified aqueous extracts; preliminary lipoid solvent extraction; inclusion of photolyzed extracts of the product in the riboflavin assay medium, or inclusion of acid or alkaline-treated extract in the pantothenic acid assay medium. B.H.W.

747. Ethyl Alcohol from Lactose in Whey. H. H. BROWNE, Bur. Dairy Indus., U.S.D.A., Washington. Canad. Dairy and Ice Cream Jour., *21*, No. 8: 23. 1942.

Fermentation of whey lactose to ethyl alcohol by three organisms, *Torula cremoris, Torulopsis sphaerica*, and *Torula lactosa*, is described. The percentage of the theoretical yield varied from 25.3 to 83.6 with the different cultures used. The economic practicability of fermenting the lactose in whey will depend upon local operating conditions. The sulfite liquors used in the manufacture of ethyl alcohol do not average over three per cent of fermentable sugars while whey contains not less than 4.5% of lactose. While the cost of whey assembled for fermentation is uncertain, it is probable that its advantage over molasses would be in the lower cost of raw materials. Its disadvantage is in the higher steam cost which is about three times that of distilling a molasses mash. O F.G.

CONCENTRATED AND DRY MILK: BY-PRODUCTS

748. Preventing Off-Flavors in Dried Whole Milk. E. L. JACK AND J. L. HENDERSON. Food Indus., *14*, No. 3: 50–52. 1942.

Dried whole milk has been increasing in its use but evaporated still exceeds it. One reason for its scant production is its low keeping quality. Within 60–90 days after production it develops a tallowy taste due to oxidation of fat. Experiments show that dry whole milk, made by the atmospheric drum process, keeps better. Low temperatures aid also.

This article goes into detail on the Buflovak Experimental Dryer, gives data, results, summary and references to dried whole milk studies.

J.C.M.

749. **Milk Solids and Bread Enrichment.** H. H. MITCHELL, T. S. HAMILTON, AND J. B. SHIELDS, Univ. Ill., Urbana. Natl. Butter and Cheese Jour., *33*, No. 9: 12. Sept., 1942.

In the manufacture of patent white flour some of the calcium, phosphorus, iron, thiamin, niacin and riboflavin are lost. When these substances are added to the flour for bread making, the flour and the bread are said to be "enriched." Feeding tests with rats show that 6% milk solids used in white bread markedly improved its nutritive value. Whole wheat bread and enriched bread with 6% milk solids excelled enriched bread with added calcium and riboflavin. Smaller amounts of whole wheat bread with 6% milk solids were required to produce definite gains in weight as compared with whole wheat bread and white bread with 6% milk solids. The presence of milk solids improved bone formation; increased riboflavin in the body, as did whole wheat bread, and improved retention of niacin; but does not improve concentration of blood hemoglobin over enriched bread or whole wheat bread; and does not maintain the thiamin content as do these two latter types of bread. W.V.P.

DISEASE

750. **Homogenized Sulfanilamide-in-Oil Intramammary Injections in Bovine Mastitis.** J. C. KAKAVAS, C. C. PALMER, JAMES R. HAY, AND EDWARD S. BIDDLE, Newark, Delaware. Amer. Jour. Vet. Res., *3*, No. 8: 274. July, 1942.

In vitro studies with several strains of *Str. agalactiae* revealed that a concentration of 20 mg. per cent of sulfanilamide, which is near the limit of tolerance, did not destroy the organisms in 70 hours at 37° C. (98.6° F.), but effectively destroyed them at 40.5° C. (104.9° F.). Different strains resisted from 0.1 to 1.0% concentration at 37° C.

A 38% suspension of homogenized sulfanilamide in a light, liquid petrolatum was injected into individual quarters in 40 cc. doses at 24-hour intervals until four injections had been given. Larger quarters were given 80-cc. initial doses. Repeated treatment was administered when indicated. Of 100 cows injected with *Str. agalactiae* and three with *Str. uberis* in a total of 265 quarters, 89.3% of the cows and 94.7% of the quarters were freed of infection including all infected with *Str. uberis*.

Sulfanilamide concentration in the milk was high, but remained low in the blood taken from the subcutaneous abdominal vein. Treatment was carried out at any time and there were no contraindications and no reactions to the treatment.

Sulfanilamide in oil in 80-cc. doses was effective against pathogenic strains of *Staph. aureus* unless udder necrosis had developed. Sulfathiazole in oil was also effective in smaller doses, but caused formation of casein curd which was difficult to remove from the udder. S.A.F.

751. The Relation of Flies (*Musca Domestica Linnaeus*) to the Transmission of Bovine Mastitis. H. E. Ewing, Jr., Newark, Del. Amer. Jour. Vet. Res., *3,* No. 8: 295. July, 1942.

With the methods used, it was found that all *Musca domestica* Linn. examined carried *Str. agalactiae* on their external surface for two days, 20% for three days, 6% for seven days and none for eight days. Some flies carried the organism internally for 10 but not for 14 days. Of seventeen cows carrying either *Str. agalactiae* or pathogenic staphylococcic infection, 16 showed the presence of the organism on at least one of the external surfaces of the teat sphincters immediately before milking, but none were found on the sphincters examined after the cows had been milked and stripped by machine.

Flies were rarely found feeding at the orifice of the teat. It is suggested that likelihood of fly transmission is greater when milk containing the organisms is present on the floor and fundamental sanitation would reduce this possibility. S.A.F.

FEEDS AND FEEDING

752. Feeding of Cows Controls Vitamin A Content of Butter. S. M. Hauge. Food Indus., *14,* No. 2: 48–49 1942.

Color is not a true index to the vitamin content of butter. Guernsey and Jersey butters are high in carotene and low in vitamin A; the reverse is true of Ayrshire and Holstein milk and butter.

Winter butters are lower in vitamin A when compared to summer butters. The problem of feeding to maintain vitamin levels in winter butters is difficult. This is due to the lack of hays and silage of excellent quality.

Butter manufacturers will not reduce the vitamin A content of butter when following orthodox procedures. Oxidation reduces the vitamin A contents of butters. J.C.M.

ICE CREAM

753. Ice Cream Vital to Farm Economy. R. C. Hibben, Exec. Sec., Internatl. Assoc. Ice Cream Mfrs., Barr Bldg., Washington. Ice Cream Trade Jour., *38,* No. 8: 24. Aug., 1942.

As proof that the ice cream industry is an essential industry from a farm standpoint, the author calls attention to the fact that this year the ice cream manufacturers will use over 6,000,000,000 pounds of milk, two-thirds of which is used during the season of peak production, when the market is needed most for the farmer. In addition to milk 30,000,000 pounds of fruit and 8,000,000 pounds of nuts are used.

Attention is also called to the fact that "Ice cream is a dairy product, a nutritious food; containing 80% by weight of milk and cream and 15%

sweetener, and with added fruit and nuts and other flavor used in ice cream, it is an important food." W.H.M.

754. Corn Syrup Solids: Their Use in Ice Cream. John W. Knechtges and H. H. Sommer, Dept. Dairy Indus., Univ. Wis., Madison, Ice Cream Trade Jour., *38*, No. 7: 14. July, 1942.

An investigation was made to determine the extent to which corn syrup solids can be used with sucrose in ice cream and the effect of such combinations on the quality of the ice cream.

Corn syrup solids lower the freezing point of mixes less than sucrose. If 4 pounds of sucrose are replaced by 5 pounds of corn syrup solids (1% higher total solids in mix) the freezing points are practically alike. If the total solids content of the mix is kept uniform by cutting the serum solids content, 4 pounds of sucrose may be replaced by 6 pounds corn syrup solids without any practical difference in freezing point.

Corn syrup solids, while acid in reaction, have such a low buffer capacity that they do not affect the acidity of the mix to any significant degree.

The extent to which sucrose may be replaced by corn syrup solids is from 25 to 33⅓% as judged by taste, body, texture and melting behavior of the ice cream.

The whipping ability of mixes is not affected by replacement of sucrose by corn syrup solids.

As judged by melt-down tests, corn syrup solids do not exert a stabilizer sparing action in ice creams with moderate stabilizer content, but in heavily stabilized ice creams the stabilizer content may have to be reduced as much as 25% when 4% of the sucrose is replaced by 5% corn syrup solids.

While slightly favorable effects in delaying sandiness and shrinkage have been observed for corn syrup solids, these effects are too slight to be of practical significance.

In comparisons of 16% sucrose ice cream with 12% sucrose plus 5% corn syrup solids ice cream by consumer preference tests no significant preference was found. W.H.M.

755. Conserving Sugar in Chocolate Ice Cream. B. I. Masurovsky, Res. Editor. Ice Cream Trade Jour., *38*, No. 6: 25. June, 1942.

The rationing of cane and beet sugar has caused ice cream manufacturers to use other available sweeteners such as corn sugar, corn syrup solids, honey, malt syrup, maple syrup, sorghum syrup and molasses. Malt syrup which contains 25% moisture, 65% reducing sugars calculated as maltose, 9% dextrine and 1% ash is recommended for use in chocolate ice cream to replace 25% of the sugar of the mix. A good grade of molasses could also be used. In this case it would take 100 pounds of molasses to replace 60 pounds of cane sugar. W.H.M.

756. Wartime Production Problems. P. H. TRACY, Univ. Ill., Urbana. Ice Cream Trade Jour., *38*, No. 8: 12. Aug., 1942.

A number of new problems have been presented to the operators of dairy plants as a result of the war. One of the most serious problems which seems to be common to all plants is the one of labor shortage. Higher wages paid to skilled laborers by defense industries, fewer college graduates and the induction of men into military service have made serious inroads into the labor supply. Milk productions have been increased in response to the demand for evaporated milk, cheese and milk powder, and in many instances this increased flow of milk has resulted in plants being operated at peak capacity which puts an additional strain on plant equipment. Due to a shortage of farm labor it has been difficult to maintain quality of the new material.

The rationing of scarce materials and supplies has forced the ice cream industry to operate on a reduced volume of sugar and chocolate and delivery services have been curtailed to meet the demands created by the rubber shortage. Market milk plants likewise have had to make many adjustments in their delivery services to meet the rubber and labor shortage.

Some operators, even with an increased volume of business, are finding it difficult, in face of higher wages, increased taxes and ceiling prices on some products, to operate their plants profitably. W H.M.

757. Chemical Sterilization of Equipment. B. I. MASUROVSKY. Ice Cream Trade Jour., *38*, No. 9: 29. Sept., 1942.

The rationing of fuel oil will necessitate some ice cream plants to reduce the amount of steam used. Since a large percentage of steam in an ice cream plant is used for sterilizing purposes, the use of chemical sterilizers offers a means of reducing the steam and therefore the oil requirements.

Chlorine sterilizers have been in common use in creameries for many years and their use is safe, convenient, and effective. The shortage of fuel further promotes their use.

In using chlorine sterilizers, the fundamental basis of success is cleanliness before the sterilizing agent is applied, since organic materials, present as grease and dirt, readily inactivate the chlorine. The increasing use of "wetting agents" in cleansing compounds and sterilizing agents helps prevent waste of chlorine. One hundred parts per million of active chlorine, by weight, are sufficient in a solution used to sterilize freezer, pipe lines, vats and average ice cream plant equipment. A new chloride disinfectant is on the market and it may prove to be another step forward in chemical sterilization. This product known as Roccol, alkyl-dimethyl-benzyl-ammonium-chloride, (1–5000 or 200 p.p.m.) is suggested for use in the sterilization of wiping cloths, to avoid transmission of micro-organisms to frozen products. W.H.M.

758. Experiments in Freezing Cream for Storage. G. M. TROUT, Mich. Agr. Expt. Sta., East Lansing, Mich. Canad. Dairy and Ice Cream Jour., *21*, No. 7: 22. 1942.

The purpose of freezing cream is largely to utilize without churning surplus cream at peak of production for later use when fat production is not so great, to conserve fat in plants which do not have facilities for churning, and to provide a more economical fat supply inasmuch as a lower price fat is being stored and that fat losses are reduced to a minimum since churning losses are not involved. The two major problems of freezing cream are the preservation of good flavor and the prevention of de-emulsification of the fat. Cream intended for freezing should have a high fat content, an excellent flavor as the cream after storage will never have a flavor superior to that when fresh, and a low titratable acidity with no developed acidity. It should preferably be high in carotene because such cream is high in color and rich in vitamin A. Results of studies indicate that cream to be stored frozen must be pasteurized at an exposure of not less than 165° F. for 15 minutes and preferably at 185° F. for five minutes. Homogenization had very little inhibitory action toward oxidation. The type of container in which cream may be stored is of little consequence so far as off flavor development was concerned. No method was found whereby the fat emulsion remained completely stabilized upon freezing. Only high-fat, excellent quality, low-acid cream, free from copper contamination should be considered for freezing. Pasteurizing such cream at a high temperature is to be preferred over low-temperature pasteurization. O.F.G.

759. The Proper Care and Use of Homogenizers in Making Ice Cream Mix. A. W. FARRALL, The Creamery Package Mfg. Co., Chicago. Canad. Dairy and Ice Cream Jour., *21*, No. 6: 26. 1942.

Homogenization breaks up fat globules into smaller sizes and has an effect on protein stability and water binding power of the ice cream mix. Recent studies show that greater protein stability is obtained when the fat breakup is secured without excessive pressures. The proper homogenizing pressure to use will depend upon the composition of the mix, the quality of the raw materials, the type of homogenizer, and other factors. The homogenizer should give a uniform steady pressure, which means that the suction and discharge valves must be kept in good condition. There must be no air leaks. Plunger packing should be removed daily for cleaning and when put in place again should be tightened only sufficiently to prevent leaks. The homogenizing valve requires periodic resurfacing. The drive end of the homogenizer usually requires only periodic change of lubricating oil. The homogenizer should be completely dismantled and washed each day in use. Care of the homogenizer is for the purpose of keeping the machine clean and maintaining it in efficient working condition. O.F.G.

760. **Reducing Oiling off of Frozen Cream Used in Ice Cream.** J. C. HENNING, Div. Dairying, N. Y. Agr. Expt. Sta., Geneva. Ice Cream Trade Jour., *38*, No. 7: 20. July, 1942.

It has been shown that homogenization alone and homogenization and the addition of gelatin did not prevent destabilization and subsequent oiling off by increasing the viscosity and clumping of the fat globules of the cream before storage. The cream separated from pasteurized milk which had been cooled to 40° F., held at that temperature for 20–24 hours, warmed to 80°–85° F. and separated did not oil off as much as cream separated from pasteurized milk which had been cooled to 80°–90° F. The cream from the treated milk oiled off less than the regular cream which had 10% sugar added. It was concluded that differences in the physical state of the cream other than the increase in viscosity was responsible for differences in oiling off after freezing. W.H.M.

MILK

761. **Sanitation of Milk Plant Operations Pays Dividends.** JAMES A. TOBEY, Amer. Inst. Baking, N. Y. Jour. Milk Technol., *5*, No. 4: 215. July-Aug., 1942.

The cost of sanitation is only a very small fraction of the cost of production and distribution. On the farm the principal cost of milk production is for feed and labor, which are nearly two-thirds of the total cost. The distributors' largest items of expense are his labor and payment to the farmer for the milk. These items represent about 70% of the dealers' total cost.

In Michigan a study made on 499 farms during 1932–1936 reported that the cost of producing milk per cow per year for market milk purposes was $1.22 more than for condensery milk.

In a more recent study made by a large dairy concern it was reported that on 376 farms in eleven markets it cost slightly more than 33 cents a hundred pounds to produce market milk complying with the milk ordinances in force as compared with the cost of producing manufactured milk; in this case evaporated. About 24¢ of this cost was for ordinance compliance and nine cents for extra transportation. Everlasting vigilance in the hygienic production of milk is necessary; many have been the penalties of poor sanitation. A number of examples are cited where courts have awarded damages to consumers due to illness caused by unwholesome or contaminated milk. L.H.B.

762. **The Administrative Public Health Problems in Milk and Milk Products.** LLOYD ARNOLD, Dept. Bact. and Pub. Health, Col. Med., Univ. Ill., Chicago, Ill. Jour. Milk Technol., *5*, No. 4: 229. July–Aug., 1942.

"Healthy cows, cleanliness and proper cooling are the underlying factors in farm sanitation. Every administrative office should separate the commercial advantages of increasing keeping qualities versus the protection of the health of the public."

Improvement in rural sanitation has been so marked during the past two decades that it is now possible to shift our administrative control from the farm to the plants. "The facilities for proper milk production may be ascertained by farm inspection which can be made less frequently than when we attempted to control the sanitary quality of the milk on the farm."

Present regulations regarding pasteurization should be continued. More emphasis should be placed on post pasteurization handling of the milk, and better packaging is desirable. "The less inspection and regulation we can do to insure a wholesome, sanitary and drinkable milk, the better off we are in the long run." L.H.B.

763. The Freezing Point of Milk. I. The Freezing Point and Solids-Not-Fat Content of the Milk of Individual Cows Throughout a Period of Lactation. R. Aschaffenburg and P. L. Temple. Natl. Inst. Res. in Dairying, Univ. Reading, Eng. Jour. Dairy Res., *12*, 315–321. 1941.

The freezing point and solids-not-fat content of individual samples of morning and evening milk from three Dairy Shorthorn cows were determined throughout the greater part of a lactation (March-Oct., 1936). The average values of the freezing points for the three cows were – 0.546, – 0.546 and – 0.544° C. (31.0172, 31.0172 and 31.0208° F.) for the morning milk and 0.548, – 0.548 and – 0.546° C. (31.0136, 31.0136 and 31.0172° F.) for the evening milk. The corresponding averages for the solids-not-fat were 8.84, 8.64 and 8.19% for the morning milk and 8.91, 8.04 and 8.14% for the evening milk.

For each animal the freezing point of the morning milk was on the average 0.002° C. (0.0036° F.) higher than that of the evening milk.

No freezing point higher than – 0.529° C. (31.0478° F.) or lower than – 0.560 C. (30.9920° F.) was encountered. The freezing point in general deviated no more than 2% and in the most extreme case, by no more than 3.5% from the mean of – 0.546 ± 0.002° C. (31.0172° F.). No evidence was found of any influence of the stage of lactation on the freezing point, but a slight increase persisting for some weeks occurred coincident with the time at which spring pasture became available to the animals. S.T.C.

764. The Plate Count and Methylene-Blue Reduction Test Applied to Milk. H. Barkworth, South Eastern Agr. Col., Whe, Kent, J. O. Irving, London School of Hygiene and Trop. Med., and A. T. R. Mattick, Natl. Inst. Res. in Dairying, Univ. Reading, Eng. Jour. Dairy Res., *12*, 265–314. 1941.

The primary object of this experiment was to find whether the standards set by the modified methylene-blue reduction test (5½ hr. winter and 4½ hr. summer) approximated to those set by the plate count method (200,000 colonies per ml.) previously used. A comparison of the grading by the two methods is given as follows:

	Summer samples		*Winter samples*	
	Number	*%*	*Number*	*%*
Passed or failed both tests	1003	87.3	631	84.6
Passed plate count, failed R. T.	105	9.1	87	11.7
Passed R. T., failed plate count	41	3.6	28	3.8

A number of samples representing subsamples from a common bulk were tested in their different laboratories. The conclusion is reached that when transit hazards are eliminated, and when standard technique is adhered to rigorously the results of the plate count, the methylene-blue test and the fat test (Gerber) are comparable between different laboratories.

Samples of morning milk were tested in quintuplicate by both plate count and methylene-blue test. The methylene-blue tubes were observed every 5 minutes. One hundeed sixty-nine samples were so analyzed. Defining sensitivity as the power of discriminating significantly between milks of different bacterial constitution, the conclusion was reached that the plate count is slightly more sensitive than the methylene-blue test observed every 5 minutes and slightly more sensitive than the latter observed every half hour. Since the coefficient of variation of the logarithm of the plate count was slightly less than that of the methylene-blue test observed every 5 minutes it is considered to be the correct measure to use. A further conclusion was that two samples whose reduction times differ by 30 minutes are not significantly different. Such a difference is only significant if the result is based on the mean of duplicate tests with 5-minute observations or of triplicate tests with half-hourly observation. S.T.C.

765. The Stability of Homogenized Milk. V. B. Sullam, N. C. State Col. Milk Plant Monthly, *31*, No. 4: 23. April, 1942.

Samples of milk testing 3.5% and 4.6% were taken (1) immediately after standardization, (2) after pasteurization at 160° F., and (3) after similar pasteurization but homogenized at 2500 pounds pressure and studied for protein stability, using protein precipitants such as alcohol, acetone, several neutral salts, aluminum sulphate, aluminum chloride, lactic and hydrochloric acid. The results obtained with alcohol, acetone, and neutral salts indicated that homogenization increased the protein stability of milk whereas, results from the other chemicals used indicated no difference in stability between raw, pasteurized and homogenized milk.

Homogenized milk could be identified by adding to a 5-ml. sample of the milk under question 2 ml. of a saturated solution of common salt and 8 ml. of distilled water. A positive test shows no separation.

The conclusion is reached that no special difficulty in the manufacturing, handling, and trading of homogenized milk be expected as a result of effects upon stability. G.M.T.

766. Elasticity of Supply of Milk from Vermont Plants. III. Forecasting the Milk Supply. S. M. JOHNSON, Vt. Agr. Expt. Sta. Bul. 480. 1942.

The purpose of this study was to determine the manner in which the supply of milk in Vermont has responded to changes in various factors, and to arrive at a more satisfactory method of forecasting the milk supply. Data on (1) how changes in production are brought about, and (2) on which they are brought about may be used as a basis of prediction.

Of the first type, most valuable for purposes of prediction are data on changes in cattle numbers. The number of milch cows can be predicted with considerable accuracy a year in advance, from data on the number of one- to two-year-old heifers on hand, and on recent slaughter and interstate shipments of cows. The rate at which grain is being fed is highly correlated with current, but not with future, production, and is of little or no value as a basis for prediction of receipts.

Of the second type of data, of things which influence farmers to change the numbers of cows milked or feeding practices, and so to change the amount of milk produced and delivered to plants, the relation between milk prices and grain prices, the milk-feed price ratio, has proven to be most closely related to subsequent production, and most useful for purposes of prediction of supply.

Of the various forms and combinations of factors examined in this attempt to develop a technique for accurate forecasting of the total market supply of milk in a producing area, the group which gave the best results (Method 11, p. 58) included factors of both types. P.H.T.

767. Effect of Homogenization on the Curd Tension, Digestibility, and Keeping Quality of Milk. C. J. BABCOCK. U.S.D.A. Tech. Bul. 832. 1942.

A pressure of 2500 gives the maximum reduction in curd tension obtainable. Milk homogenized after pasteurization will have a lower curd tension if processed at a temperature either higher or lower than the temperature of pasteurization. There is no advantage of the two-stage homogenizer over the single-stage machine as far as curd-tension reduction is concerned. An increase in butterfat results in a reduced curd tension. A mixture of homogenized and unhomogenized milk produces a product with a curd tension slightly lower than the theoretical average curd tension for that particular combination. Partial freezing does not materially effect the curd tension of milk.

By digestion in vitro, it was found that proteolysis takes place more rapidly in either boiled or homogenized milk than in raw milk but not more completely.

In the presence of artificial gastric juice homogenized milk forms smaller curds than unhomogenized milk.

Homogenization retards or inhibits the development of copper induced oxidized flavor in milk. Approximately 10 times as much copper is required to produce an oxidized flavor in homogenized milk as is required to produce a similar flavor in the same milk unhomogenized.

Homogenized milk is more susceptible to the action of sunlight than is unhomogenized milk.

From a bacteriological standpoint (as determined by the plate count) there is no significant difference in homogenized and unhomogenized milk. The extent of acid development in those two types of milks is similar

P.H.T.

768. The Bacterial Population of Paper Milk Containers in Relation to Methods of Moisture Proofing. J. W. Rice, N. Y. Agr. Expt. Sta., Geneva. Tech. Bul. 263. 1942.

Thirty-two hundred Pure Pak milk containers were made up and sealed under commercial conditions for experimental purposes in 16 series of 200 containers each. Each series of containers received special moisture-proofing in the form of a specific melting-point paraffin applied at a given temperature. In the laboratory, under random sampling, 100 containers from each waxing treatment were subjected to nutrient broth sterility tests, and an additional 276 containers were tested by plating sterile water rinses to obtain bacterial counts. In both of these methods the sterile charges of water and of broth were introduced by a special syringe-needle-puncture method to avoid possible air contamination.

The efficiency of wax coverage obtained in the several moisture-proofing processes was studied by making microscopic examinations and counts of punctiform spots on the inside walls and by planimetric measurements of stained corners and seams on the insides of containers which were treated by 24-hour contact with a 0.1% methylene-blue solution at room temperature.

From the broth sterility tests made on a total of 1600 containers, selected uniformly from the several moisture-proofing treatments, it was found that approximately 80% of them were sterile. The temperature at which moisture-proofing was done, as well as the melting-point of the paraffin used in the various treatments, showed practically no differences in the percentage of sterility among the several sets of containers. Plate counts of viable bacteria from the platings of water rinses made on 276 containers, chosen from the moisture-proofing processes, revealed approximately 60% sterility.

Only 10 containers yielded more than five colonies per container. The moisture-proofing methods employed showed no effect on the percentage sterility nor on average colony counts per container by this method of testing.

Planimetric readings of methylene-blue-stained corners and seams and counts of punctiform spots on the inside walls of 320 containers selected uniformly from all methods of moisture-proofing indicate quite clearly that the most efficient moisture-proofing is obtained by treating paper containers with a high-melting-point wax, such as 133° to 135° F. American melting point applied at the lowest possible temperature, such as 150° F. P.H.T.

769. How Methods and Promptness of Cooling Affects the Quality of Milk. A. J. Gelpi, C. S. McCleskey, D. M. Smith, La. State Univ. Univ. Bul. 344. 1942.

The following methods of milk cooling were studied: (1) Cooling tank using 33°–40° F. water agitated. (2) Conical cooler to 33°–36° F. followed by storage in can in water tank (33°–40° F.). (3) Tubular surface cooler to 33°–36° F. followed by storage in can in water tank at 33°–40° F.

It was found that the milk cooled in the can in the tank (method 1) had the highest bacterial content. Delayed (2 hours) cooling was of no significance in the case of methods 2 and 3 but resulted in a significant increase in bacteria in the milk cooled by method 1 after holding 12 hours. There was no difference in the flavor of the milks that could be attributed to the method of cooling.

Directions for the production of high-quality milk are included.

P.H.T.

770. Bacteriological Control of Milk Quality. Lawrence Little, Sterling Meadow Gold Dairy, Oklahoma City. Jour. Milk Technol., *5*, No. 4: 221. July–Aug., 1942.

Tests were made on samples of both inspected and uninspected milks over a period of 14 months for determining the presence of thermoduric organisms.

It was found that thermoduric organisms were prevalent in both supplies. In the uninspected milks they were present the year round, while in the inspected or graded milks they were of little consequence in the summer but were a problem in the winter.

Microscopic examination of incubated samples of raw milk was of no value in detecting the presence of thermoduric organisms. Neither was the fermentation test of any value. The methylene-blue and resazurin tests were also found to be ineffective for detecting samples with large numbers of thermoduric organisms.

Laboratory pasteurization is necessary for detecting these organisms. It definitely detects a type of unsanitary conditions on the farm that is not detected by any of our present tests. However, it in itself is not a sufficient test for determining the sanitary quality of a milk supply. Meyers and Pence's oval tube technique was used in connection with the pasteurization test and proved its worth. It was found that it could replace the standard agar plate count altogether for routine control work, saving labor, equipment and space. L.H.B.

771. **The "Cream Top" Type Bottle for Laboratory Sampling of Homogenized Milk.** JOSEPH LEVINE AND RUBIN H. FEINGOLD, Bur. Food and Drugs, Dept. Health, N. Y. Jour. Milk Technol., *5*, No. 4: 202. July–Aug., 1942.

A simple and practical method for sampling homogenized milk for determining the fat content in the upper 100-ml. layer is described and illustrated.

Homogenized milk, when brought into the laboratory, is thoroughly mixed and then poured into the cream top bottle; placed in the refrigerator and allowed to set undisturbed for 48 hours, at end of which time a plunger-type apparatus which is illustrated in the article is inserted in the bottle so that it forms an effective seal in the neck, permitting the pouring off of the upper layer. A volume of approximately 100 ml. is obtained in this portion. L.H.B.

772. **The Influence of Ammonia on the Development of Rancidity in Milk.** C. H. CASTELL, Dept. Bact., Ontario Agr. Col., Guelph, Ontario. Jour. Milk Technol., *5*, No. 4: 195. July–Aug., 1942.

Preliminary experiments conducted showed that both addition of ammonia to milk and the exposure to an atmosphere containing ammonia were effective in accelerating the reduction in surface tension and in the development of rancid flavors when milk was held at 5° C.

It was found that there was an apparent difference in effect on the milk from different cows, and to a lesser degree with different breeds. The effect increased as the lactation period advanced.

The author doubts if the concentration of ammonia, except in extreme cases, in the atmosphere of the barn where cows are milked would ever be a major factor in causing the development of rancidity in milk and cream. However, in late winter and early spring a strong odor of ammonia is not uncommon in many barns, and could very well be a contributing factor. Also, a breakdown of a refrigeration unit, accompanied by an escape of gas in the atmosphere, could be a factor in accelerating the development of rancidity in raw milk and cream. L.H.B.

773. **Thermoduric Organisms in Relation to High-Temperature Short-Time Pasteurization.** F. W. FABIAN, Res. Prof. Bact., Dept. Bact. and Hygiene, Mich. State Col., East Lansing. Jour. Milk Technol., *5*, No. 4: 237. July–Aug., 1942.

"Thermoduric in dairy bacteriology is used to designate a group of bacteria which will withstand the temperature of milk pasteurization, 140 to 145° F. for 30 minutes, or 160–161° F. for 15 to 16 seconds, but will not. grow at this temperature."

We should expect to find more thermophilic bacteria in milk pasteurized at 142–145° F. for 30 minutes than in milk pasteurized at 160–161° F. for 15–16 seconds, and conversely we should expect to find more thermoduric bacteria in milk pasteurized at 160–161° F. for 15–16 seconds than in milk pasteurized at 142–145° F. for 30 minutes.

Four groups of these latter mentioned organisms are commonly found in milk, being micrococci, streptococci, sarcinae and bacilli. Most common are the micrococci.

The principal sources of these organisms are the udder, poorly cleaned and improperly sterilized milking machines, milk pails, cans and other milk utensils.

Methods most generally used for determining the presence of thermoduric bacteria are laboratory pasteurization of the milk samples, then plating, or laboratory pasteurization at 161° F. for 16 seconds, then incubating at 37° C. for seven hours, after which they are examined under the microscope. Another method is to incubate the raw milk at 58° to 60° F. for two hours and then examine under the microscope.

Thermoduric bacterial control is a producers' problem. L.H.B.

774. **Problems Incident to the Production and Use of Homogenized Milk.** G. M. Trout, Mich. Agr. Expt. Sta., East Lansing, Mich. Jour. Milk Technol., *5*, No. 4: 233. July–Aug., 1942.

The problems involved are processing, packaging, distribution, laboratory control, cooking and utilization of returns.

Processing creates problems which require attention, such as processing temperature, clarification, bacterial counts, cream line and efficiency of homogenization treatment in order to meet required standards.

Packaging problems are created due to the fact that homogenization increases foaming, making it difficult to fill the packages properly.

Distribution problems encountered are seepage around cap seat in the summer, and in winter, watery appearance of the upper portion when thawed after freezing.

Laboratory control: Modification of the Babcock test is required. H_2SO_4 acid, having a sp. gr. of 1.815–1.820, is recommended.

Efficiency of homogenization must be checked.

Cooking: Problems arising in the use of homogenized milk in cooking deal chiefly with the fact that it is apparently more sensitive to heat than unhomogenized milk.

Utilization of returns: This is not so much of a problem as when homogenization was first introduced. It has been shown that homogenized milk can be successfully used in making cottage cheese and buttermilk. L.H.B.

775. **Merits of Churned Buttermilk.** C. L. Roadhouse, Univ. Calif., Davis, Calif. Canad. Dairy and Ice Cream Jour., *21,* No. 8: 54. 1942.

The following points are suggested for making a churned buttermilk that is pleasing in flavor, does not "whey off" readily, contains butter granules, and between 1 and 2% fat: (1) Use a clean-flavored vigorous starter, (2) Select skim milk of the best quality, (3) Pasteurize so as to destroy as many bacteria as possible, (4) Pasteurize the cream separately at 145° F. for 30 minutes, (5) Mix the cream with the skim milk before or after the starter is added, (6) Add 0.75% fat in the form of cream, (7) Develop an acidity of 0.7 to 0.8%, (8) Add a small amount of salt to improve the flavor, (9) Churn at a temperature between 68 and 72° F., and (10) add to the churned buttermilk at least 0.025% sweet cream. O.F.G.

776. **Adjusting Milk Delivery to Meet Tire Shortage.** W. P. Mortenson, Univ. Wis., Madison, Wis. Canad. Dairy and Ice Cream Jour., *21,* No. 8: 29. 1942.

This is a timely article dealing with ways and means of conserving tires in the dairy industry. The author suggests that the following services can be discontinued: (1) Collecting milk accounts and soliciting new customers, (2) Special delivery, and (3) Special brands of milk and specialty products. Other services can be materially reduced and the author suggests the following five plans: (1) Zone the city for home milk delivery, (2) Form an independent unified delivery system for the entire market, (3) Every other day milk delivery, (4) Eliminate home delivery entirely and let consumers obtain milk from retail stores and milk depots, (5) Unify the whole milk distribution system, that is (a) set it up as some kind of public utility like gas, street car, bus, electric system, or (b) let the city own the milk distribution as it now does the water system.

None of these plans will bring about a complete solution satisfactory to all interests in the market. O.F.G.

777. **Canadian Agriculture in the Post-War Period.** J. F. Booth, Assoc. Dir. of Marketing, Dept. Agr., Ottawa, Canada. Canad. Dairy and Ice Cream Jour., *21,* No. 5: 30. 1942.

A 200-year chart of price levels shows a gradual improvement of farm prices relative to general wholesale prices. In each war period, prices increased more than 100% and in each case they returned to a level close to, or below, that prevailing before the war. There is certain evidence that agriculture has been adversely affected by the outcome of wars. An increase or decrease in retail prices of 20% may mean a 30% change in wholesale prices and a 50% change in farm prices. Such a relationship indicates that farmers are much more drastically affected by price changes than most other groups. This characteristic of farm prices is closely related to a failure of farmers to quickly adjust production levels after a period of inflation and high production level. The author's interpretation of past records brings the conclusion that wartime inflationary prices have been distinctly harmful to agriculture. Farmers have every reason to join with others in a program of price stabilization. The post-war period will be influenced by demobilization and soldier settlements, adjustments in industry and labor, international trade relations, immigration policies, and reconstruction abroad. Farmers should not oppose a policy that involves the admission of immigrants representing various occupations in somewhat the same proportion as such occupations are now represented in the Canadian population. The question of markets in the post-war period is of great importance to Canadian agriculture and most economists favor the removal of trade barriers in order to increase these markets. The opportunities of expanding the domestic Canadian market for certain foods is good since a substantial proportion of Canadian people do not obtain a sufficient supply of certain foods. O.F.G.

778. **A New Order in the Making for Canadian Dairymen.** J. E. LATTIMER. Canad. Dairy and Ice Cream Jour., *21*, No. 4: 25. 1942.

This is an address given before the National Dairy Council of Canada. The author points out that Canada ranks high in per capita consumption of dairy products because of its large consumption of butter. Canadians in general pay less for their fluid milk than do United States consumers. The provision of cheap milk by the producer is primarily due to the great number of milk cows in proportion to population in Canada, the small dependence on hired labor, and the ability and persistence of the dairy farmer. Price regulation of fluid milk has come into existence primarily to insure a regular supply to the consumer. Regulation has been extended recently to cheese and butter. If a new order for the farmer in general and the dairy farmer in particular is wanted, the first need is a new deal in the matter of wages. Past records indicate that a new order is necessary. The following points must be considered in establishing a new order for the dairy farmer: 1. Decentralization of butter making, 2. Reduced costs of distribution of fluid milk, 3. Raising dairy heifers in less expensive surroundings, 4. Artificial insemination, 5. Improved pastures, 6. Increased volume of production per farm, 7. Cheaper feeds. O.F.G.

PHYSIOLOGY

779. **Studies on Experimental Teat and Mammary Development and Lactation in the Goat.** S. J. FOLLEY, HELEN M. SCOTT WASTON,

AND A. C. BOTTOMLEY. Natl. Inst. Res. in Dairying, Univ. Reading, England. Jour. Dairy Res., *12,* 241–264. 1941.

The authors summarize their results as follows:

1. Observations on five animals indicate that the teats of immature castrated male goats grow isometrically. Less extensive data on three animals allow of the tentative conclusion that the same is true of immature normal males. In the male goat, therefore, teat growth appears to be uninfluenced by the testes.

2. Administration of diethylstilbestrol or its dipropionate to normal or castrated immature male goats causes the teats to grow allometrically for a time.

3. No externally visible udder growth occurred even when oestrogen treatment was prolonged for periods of over a year and was supplemented by progesterone or ethinyltestosterone. Whole mounts of glands from treated animals indicated that some mammary growth had occurred. In two cases microscopic examination showed the presence of alveoli. No explanation can be offered of the failure to develop the udder in the male goat experimentally.

4. Endocrine activity of the ovary as evidenced by a change from isometric to allometric teat growth often manifests itself in the young female goat at an early age. In one case allometric teat growth was in progress at 41 days of age. During the allometric phase the data agree with the simple allometric law.

5. During the rutting season following its birth, teat growth ceases completely in the female goat; allometric growth is resumed when the rutting season ends. It therefore appears that the corpus luteum inhibits teat growth.

6. Administration of diethylstilbesterol or its dipropionate (by inunction of the udder region) causes, in the virgin female, an increase in the rate of teat growth accompanied by udder growth.

7. In virgin females the above treatment was followed, after a latent period during which udder growth occurred, by prolonged lactation, at the height of which the daily milk yield was of the order of 1 5–2 1. No treatment with anterior pituitary extract was necessary. In normal or castrated males the treatment resulted in the secretion of only minute quantities of milk.

8. Lactation was initiated and increased in intensity while oestrogen administration continued. It is thought that low doses of oestrogen exert a galactopoietic effect through the anterior pituitary.

9. During the first part of the artificial lactation period, the changes in milk composition were similar to those characteristic of the colostral period. Subsequently, milk of excellent chemical quality was secreted. S.T.C.

780. **Effects of Testosterone Propionate and Stilbestrol on the Mammary Gland Postpartum.** Edward M. Jeppson, Harry Y. Kasabach and Aaron E. Kanter, Salt Lake City and Chicago. Jour. Clin. Endocrinol. *2*, No. 1: 16. Jan., 1942.

A study was made of 124 patients to determine the value of testosterone propionate in the prevention of pain of breast engorgement in the puerperium. The results showed that testosterone propionate was of little or no value for this purpose and of some disadvantage since it caused delay and discontinuance of lactation. Testosterone and diethylstilbestrol were useful in preventing or discontinuing lactation. R.P.R.

781. **Use of Ethinyl Estradiol to Prevent Lactation in Puerperal Patients.** Lawrence Kurzrok, Charles H. Birnberg, and Seymour Livingston, Jewish Hospital and Greenpoint Hospital, Brooklyn, N. Y. Jour. Clin. Endocrinol., *2*, No. 7: 471. July, 1942.

Fifty-nine puerperal women were given ethinyl estradiol orally for 3 to 4 days. When amounts up to 1.5 mg. were given daily the results were only fair, with 9 failures in a series of 26 cases. Excellent results were obtained in 12 cases when 1.5 to 2.4 mg. of ethinyl estradiol were given daily. There was one failure. The administration of 1.8 to 2.4 mg. daily for 3 to 4 days, 4 to 21 days post-partum, inhibited lactation even though the women had begun to nurse their babies. R.P.R.

782. **Comparative Clinical Effects of Orally Administered Alpha-Estradiol and Diethylstilbestrol on Post-partum Engorgement of the Breast.** A. W. Diddle, S. F. Nagyfy, and R. L. Sells, State Univ. of Iowa. Jour. Clin. Endocrinol., *2*, No. 5: 307. May, 1942.

Sixty-one post-partum women were given alpha-estradiol in propylene glycol sublingually to test its effect on breast engorgement as compared with that produced by diethylstilbestrol. Comparable dosages of both drugs given over the same period produced similar results provided the alpha-estradiol was given in divided doses at regular intervals throughout the day and night. Initiation of lactation was usually delayed but not inhibited entirely. R.P.R.

783. **Selection of Nipples by Suckling Rats and Its Effect upon Mammary System.** Charles K. Weichert, Univ. Cincinnati. Endocrinol., *31*, No. 3: 349. Sept., 1942.

Observations on 67 lactating rats showed that when small litters were being suckled not all teats were used by the young. In only one of over 150 animals suckling 7 or more young was there neglect of any teats. There was a correlation between litter size and the specific teats which were utilized. Selection of teats took place in an anterior-posterior sequence, litters of one confining themselves to the anterior pectoral region. The mammary parenchyma of neglected teats retrogressed while that associated with suckled teats was fully functional. Prolactin injections failed to maintain complete activity in neglected mammary parenchyma. The importance of careful observation of teats prior to removal of mammary tissue was emphasized. R.P.R.

784. Influence of Thyroxine upon Mammary Lobule-Alveolar Growth. JOHN P. MIXNER AND C. W. TURNER, Univ. Mo. Endocrinol., *31,* No. 3: 345. Sept., 1942.

An optimal dose of thyroxine significantly increased the percentage of ovariectomized mice which responded with mammary lobule-alveolar growth to minimal doses of progesterone plus estrone. The thyroxine administration increased the efficiency of progesterone plus estrone in stimulating mammary lobule-alveolar growth by about 25%. Thyroidectomy inhibited the ability of ovariectomized mice to respond to progesterone plus estrone with mammary lobule-alveolar growth. R.P.R.

785. Studies Concerning Mechanism Controlling Initiation of Lactation at Parturition. IV. Influence of Suckling on Lactogen Content of Pituitary of Postpartum Rabbits. JOSEPH MEITES AND C. W. TURNER, Univ. Mo. Endocrinol., *31,* No. 3: 340. Sept., 1942.

The effect of the nursing stimulus after parturition on pituitary lactogen content was determined in 72 New Zealand White rabbits either by permitting the litters to remain with their mothers or by removing them at birth. On the second day after parturition the pituitary lactogen content of nursed and non-nursed does was approximately the same. There was an abrupt increase in pituitary lactogen content to a peak level on the fifth day postpartum in both groups, however, the pituitaries of the nursed group contained 66% more lactogen than did the pituitaries from the non-nursed group. In both groups pituitary lactogen content had decreased at 10 days post-partum and this was followed by a further decrease at 20 days postpartum. Mammary secretion appeared to be fully established 5 days after parturition although considerably more milk was present in the glands of the nursed rabbits than was present in the glands of the non-nursed rabbits. R.P.R.

786. Lactogenic Hormone Content of Anterior Pituitary Gland of Albino Mouse as Compared to Other Species. VICTOR HURST AND C. W. TURNER, Univ. Mo. Endocrinol., *31,* No. 3: 334. Sept., 1942.

The lactogen content of the pituitary of the virgin female was relatively low. By the 10th day of pregnancy there was an 88% increase in pituitary lactogen content on the basis of 100 gm. of body weight. This was followed by an additional increase of 63% following parturition.

On the 5th day post-partum pituitary lactogen content was 201% above the virgin female level after which it declined rapidly. At 21 days post-partum the lactogen level was 38% above that of the virgin female. Neither ovariectomy nor thyroxin injections altered the pituitary lactogen content.

R.P.R.

787. Influence of Antagonism Phenomenon on Mammary Gland Development. Fritz Bischoff and Louise P. Ingraham, Santa Barbara Cottage Hospital Res. Inst. Endocrinol., *31,* No. 3: 326. Sept., 1942.

Injection of sheep pituitary gonadotropin once daily for 10 days into mature virgin female mice under conditions designed to produce rapid, moderately delayed, or delayed absorption produced a degree of ovarian hypertrophy proportionate to the delay in absorption. Rapid absorption of the hormone caused no significant alveolar development but delayed absorption produced marked alveolar development of the mammary gland.

R.P.R.

MISCELLANEOUS

788. Food Plants Prepare for Air Raids. Thomas Robert Edwards, Jr. Food Indus., *14,* No. 3. 1942.

Practical precautions taken by food plants for protection against air raids were studied. A coffee plant, doing no work at night, had no blackout facilities, but fire drills were practiced. A bakery was found best prepared.

The bakery had sliding panels to keep light in and flying splinters out. They have sand available and also have appointed fire wardens. They have an auxiliary electric plant and a supply of oil in case the gas goes off. The water supply is guarded in a sealed tank. Their employees have been well trained and know exactly what to do.

The dairy used in the survey also has sand available and has an underground tunnel for protection. The shift engineer has charge of throwing off the switches and the water supply is well protected.

Canneries have done little as they usually operate in rural areas. The operators interviewed were studying further plans.

Meat packers have changed their loading hours, blackout windows are installed, and more guards have been added. Their people are also well trained on what they are to do. An adequate signal system has been set up also.

J.C.M.

789. Army Needs 13 New Foods. Major Jesse H. White. Food Indus. *14*, No. 3: 38–39. 1942.

This article lists the following as desirable for the army: (1) pork sausage patties, (2) liver combinations, (3) beef and noodles, (4) pork hash has possibilities, (5) corned pork and chopped ham, (6) concentrated soups or gravies, (7) meat and spaghetti (not yet approved), (8) Hungarian goulash (from Old World recipe), (9) canned mortadella sausage meat, (10) beef and rice (so far rice not desirable), (11) cheese and bacon, cheese and ham, using both smoked and unsmoked cheese, (12) gelatin coatings for hams and cheese, (13) new packing uses: cellophane, pliofilm, Cry-O-Vac. coverings for meat and cheese to prevent loss of moisture.

J.C.M.

790. Improved Firing Cuts Fuel Bill 38%. Francis A. Westbrook. Food Indus., *14*, No. 3: 43. 1942.

Taystee Bread Co., Indianapolis, Ind., saves around $1600 a year since they installed a stoker. With this arrangement one boiler does the work that two had done previously. The pneumatic spreader stoker feeds two boilers at the same time and can be switched in a few minutes. Each boiler has a below-grate fan, pinhole grates and a feed tube into the firebox. A valve shuts off the intake of hot gas. Automatic controls are used for steam, air, etc.

The boiler room can be kept clean, as there is no manual handling of the coal. Coal too large is crushed automatically. The coal is burned more completely and ash is easier to remove. A check made in winter showed no "fly ash" on the snow around the building. J.C.M.

791. Protect Your Plants from Bombs and Sabotage. Anonymous. Food Indus., *14*, No. 2: 37–40. 1942.

This article gives details for destruction prevention after bombings and sabotage. Three documents on blackouts, civilian defense and protection of industrial plants available at a low cost from the Supt. of Documents at Washington are recommended. These can be purchased for twenty-five cents each.

A total of 26 steps are listed in keeping plants free from bomb and sabotage damage.

Functions, fire protection, medical and maintenance services are discussed in detail.

This article is too detailed for abstracting in full. Its value lies in a complete understanding of the entire article and a knowledge of the material in the documents listed as being available to all plant operators. J.C.M.

792. Preventing Spoilage by Mold and Bacteria. E. F. GLOBE. Food Indus., *14,* No. 2: 46–48. 1942.

The characteristics of molds are discussed. The four commonly used inhibitors in the bread industry are discussed as a possibility of finding applications in other industries.

The chemistry of inhibitors is important as their use in various ways is limited by their reaction. All inhibitors accepted do not effect flavor or color; two items of primary importance. J.C.M.

793. Relation of Equipment to Operating Economics. G. D. AMERDING, Mojonnier Bros., Chicago, Ill. Canad. Dairy and Ice Cream Jour., *21,* No. 8: 25. 1942.

A recent survey of 30 receiving stations showed the total operational costs divided up as follows: Waste 1%, fuel 5%, supplies 5%, maintenance 7%, administration 10%, power 11%, fixed expenses 24%, labor 37%. The following suggestions are made on how to prolong the life of equipment: (1) Do not try to save on lubricating oil, (2) Keep the equipment clean, (3) Do not crowd the equipment, (4) Do not abuse the equipment. Other points discussed are care of conveyors, care of sanitary fittings, care of rubber hose, checking of the brine system, care of the separator, disposal of acids, being economical with labor because of the shortage, conservation of electricity, full use of water, saving of fuel, care of electric motors, and care of coolers and cabinets. O.F.G.

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